

# The Integrated Field-Scale Subsurface Research Challenge Site (IFC) at Rifle, Colorado: Preliminary Results on Microbiological, Geochemical and Hydrologic Processes Controlling Iron Reduction and Uranium Mobility

P.E. Long<sup>1</sup>, J. Banfield<sup>2</sup>, R. Bush<sup>3</sup>, K. Campbell<sup>4</sup>, D.P. Chandler<sup>5</sup>, J.A. Davis<sup>4</sup>, R. Dayvault<sup>6</sup>, J. Druhan<sup>2</sup>, H. Elifantz<sup>7</sup>, A. Englert<sup>8</sup>, Y. Fang<sup>1</sup>, R. L. Hettich<sup>9</sup>, D. Holmes<sup>7</sup>, S. Hubbard<sup>8</sup>, J. Icenhower<sup>1</sup>, P.R. Jaffe<sup>10</sup>, L.J. Kerkhof<sup>11</sup>, R.K. Kukkadapu<sup>1</sup>, E. Lesher<sup>12</sup>, L. Li<sup>8</sup>, M. Lipton<sup>1</sup>, D. Lovley<sup>7</sup>, S. Morris<sup>6</sup>, S. Morrison<sup>6</sup>, P. Mouser<sup>7</sup>, D. Newcomer<sup>1</sup>, L. N'Guessan<sup>7</sup>, A. Peacock<sup>13</sup>, N. Qafoku<sup>1</sup>, C. T. Resch<sup>1</sup>, F. Spane<sup>1</sup>, B. Spalding<sup>9</sup>, C. Steefel<sup>8</sup>, N. VerBerkmoes<sup>9</sup>, M. Wilkins<sup>2</sup>, K.H. Williams<sup>8</sup>, S.B. Yabusaki<sup>1</sup>

*ERSP PI Meeting April 8, 2008, Lansdowne, VA*

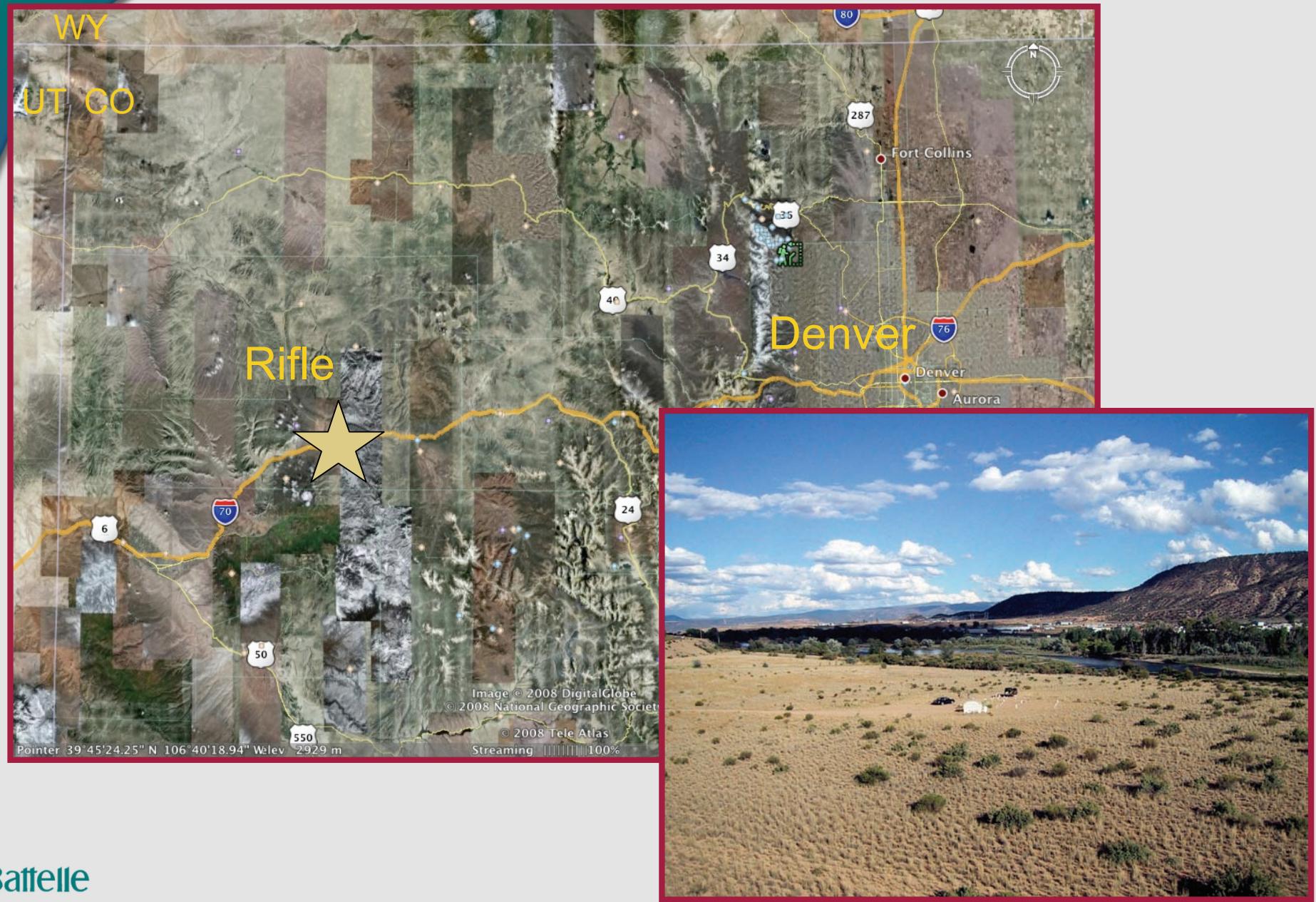
# Outline of Rifle IFC Overview

- ▶ Background and context
- ▶ Selected Rifle IFC Site Characteristics
- ▶ The Winchester Experiment, Summer 2007
- ▶ Overall status of the project and future directions

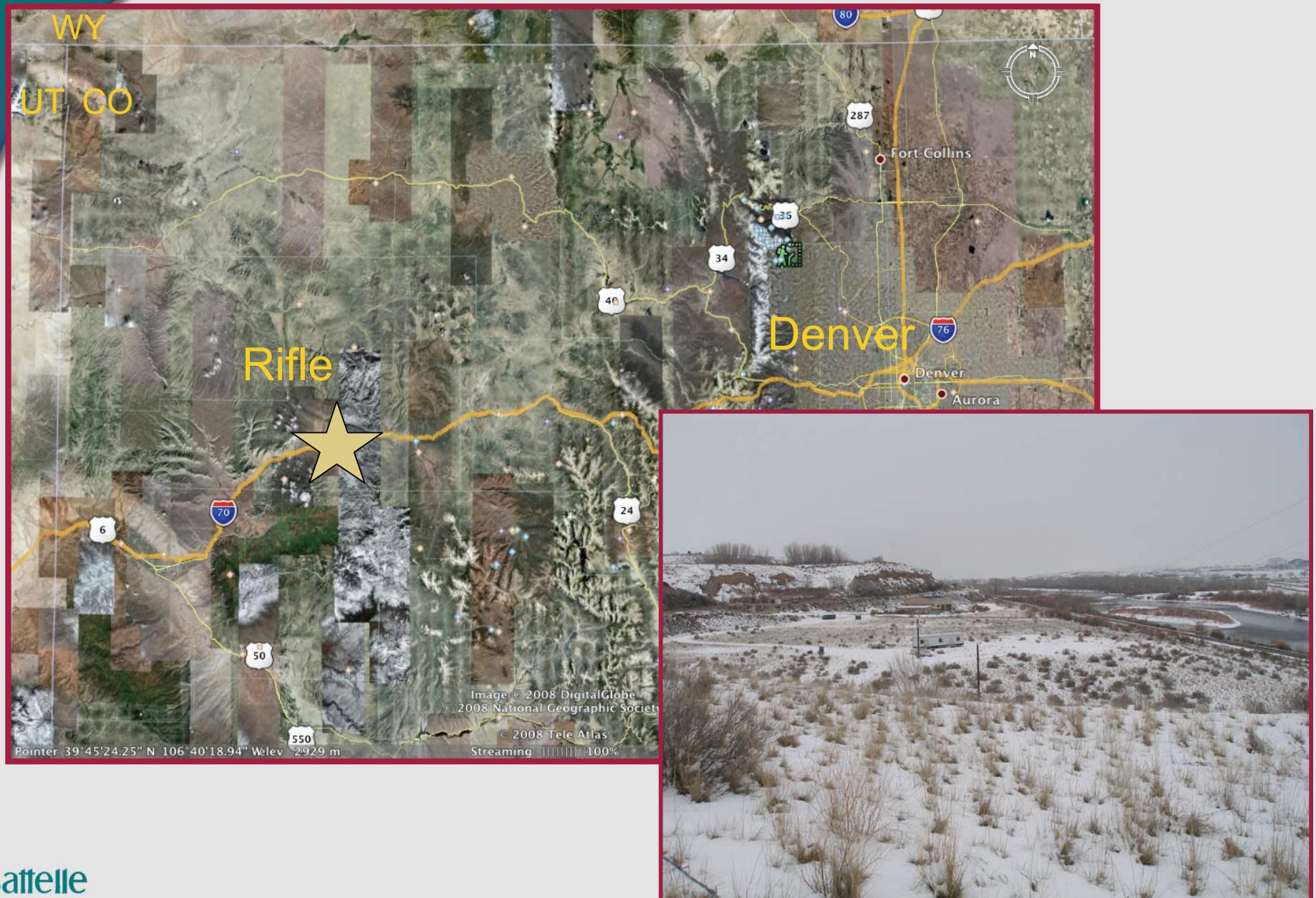
## ***Presentations on two key aspects of the Winchester Experiment:***

- ▶ Gene expression and stable Isotope probing (SIP) results (Derek Lovley, U Mass)
- ▶ Proteogenomics results (Jill Banfield, UC Berkeley)

# Rifle IFC Location



# Rifle IFC Location

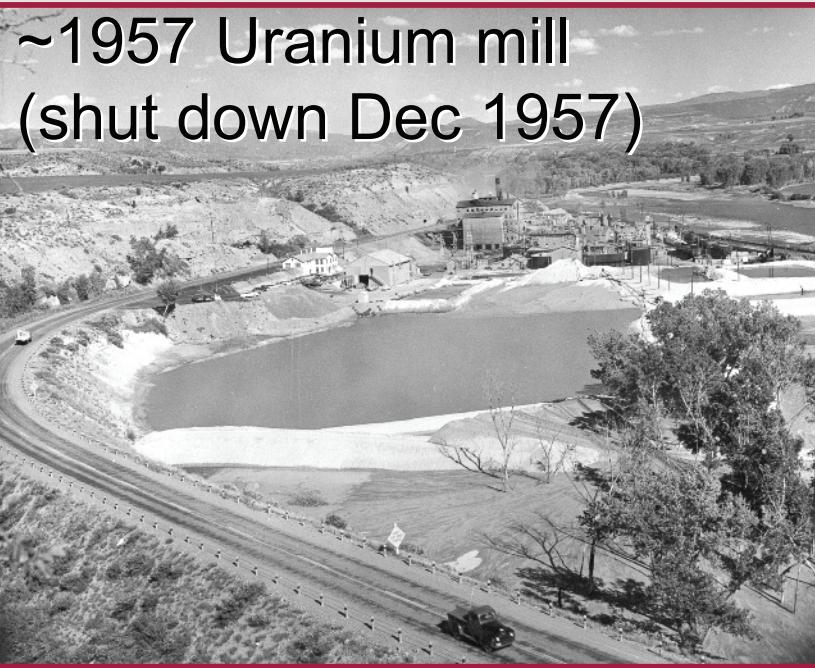


# Site History...

~1924 Original vanadium mill



~1957 Uranium mill  
(shut down Dec 1957)



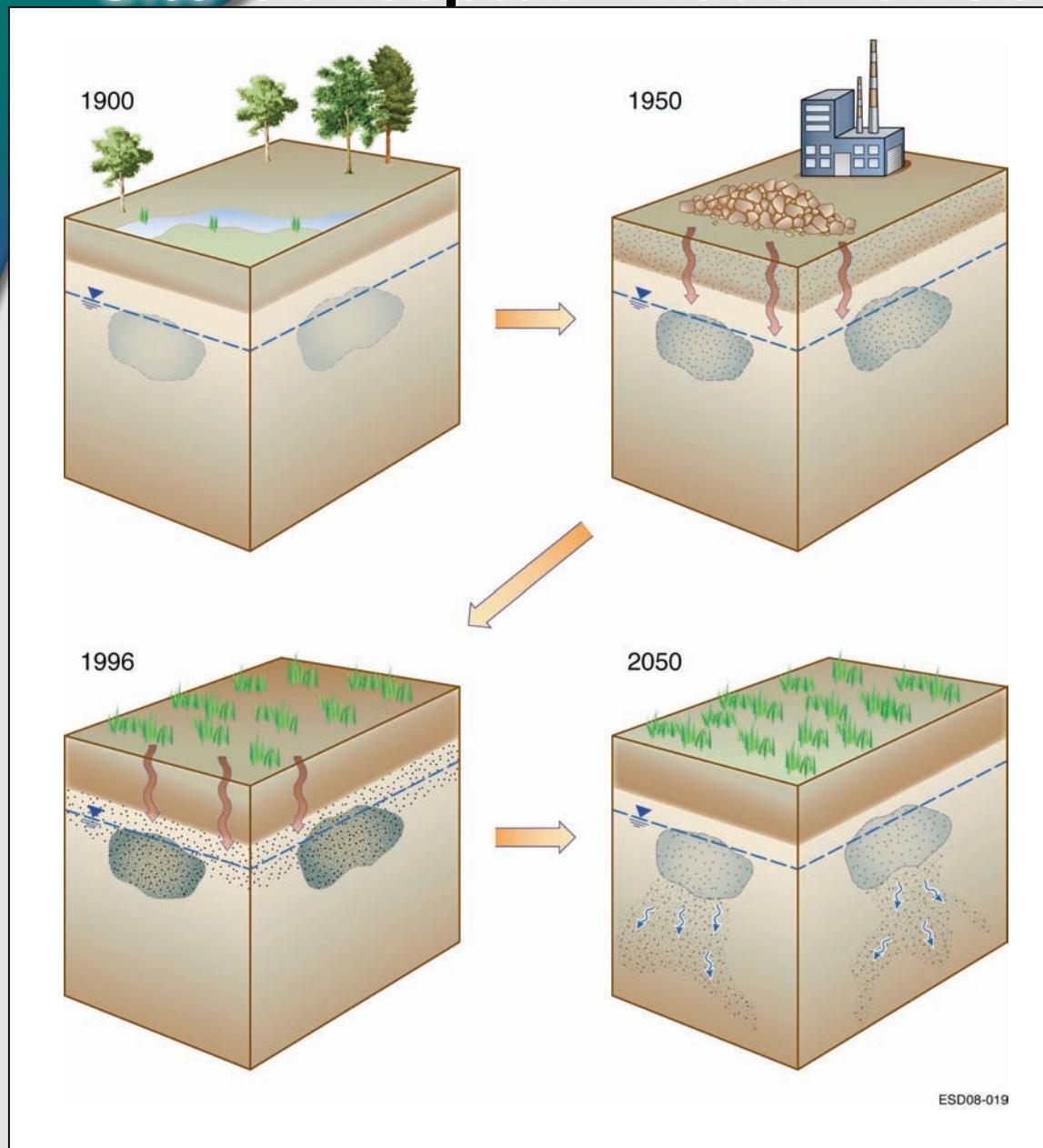
~1960



Fall 1967



# Site Conceptual Model for Contamination



## ► Importance of the Rifle IFC for DOE-LM and DOE-EM:

- Addresses large, dilute U plumes not attenuating as rapidly as predicted
- Combined biotic and abiotic mechanisms for U behavior under both natural and biostimulated conditions



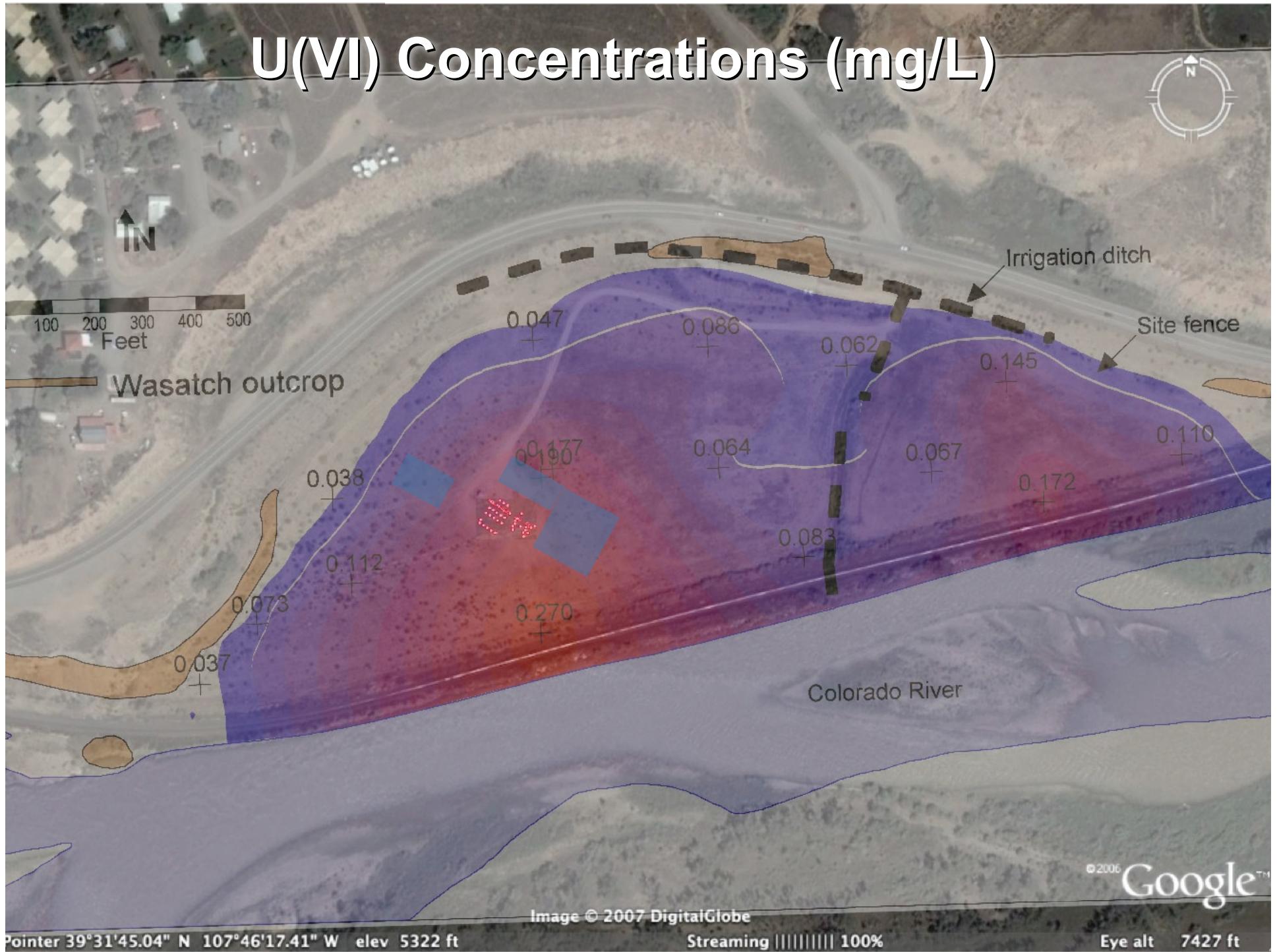
*U.S. Department of Energy Office of Science*

**Environmental Remediation Sciences Program**

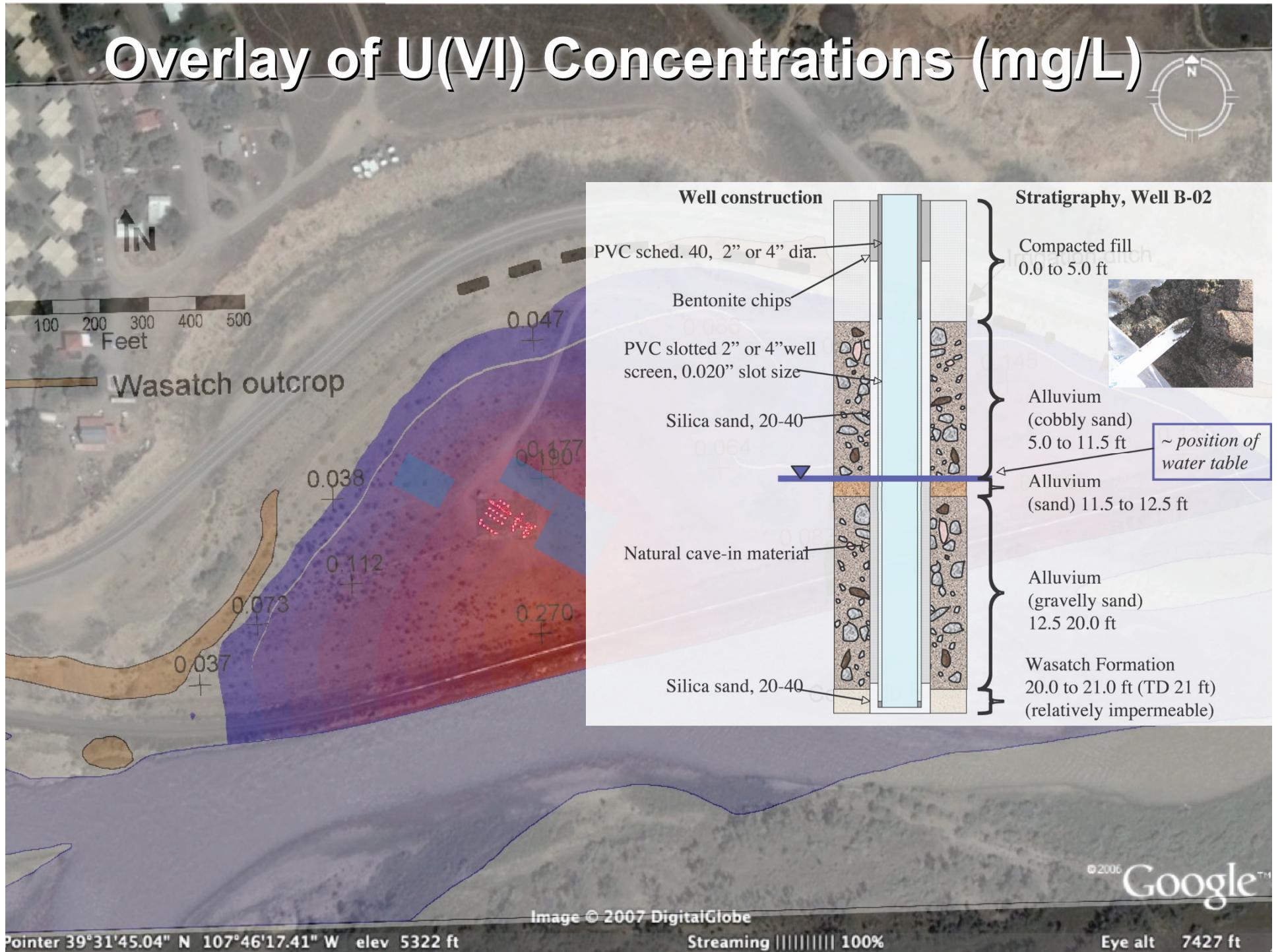
# Rifle Integrated Field Challenge Site:

- ▶ Hypotheses
  - Extension of Fe reducing conditions
  - U(VI) Sorption under reducing conditions
  - Mechanisms for post-biostimulation U removal
  - Rates of natural bioreduction of U
- ▶ Science Themes
  - How do changes in the membership and activity of microbial communities alter uranium bioreduction?
  - What are the relative contributions of biotic processes and abiotic uranium immobilization processes evaluated (e.g. U bioreduction and U sorption)?
  - How do subsurface geochemical processes correlate with geophysical monitoring of subsurface redox status associated with bioreduction?
  - Development of comprehensive reactive transport modeling of uranium mobility in the subsurface

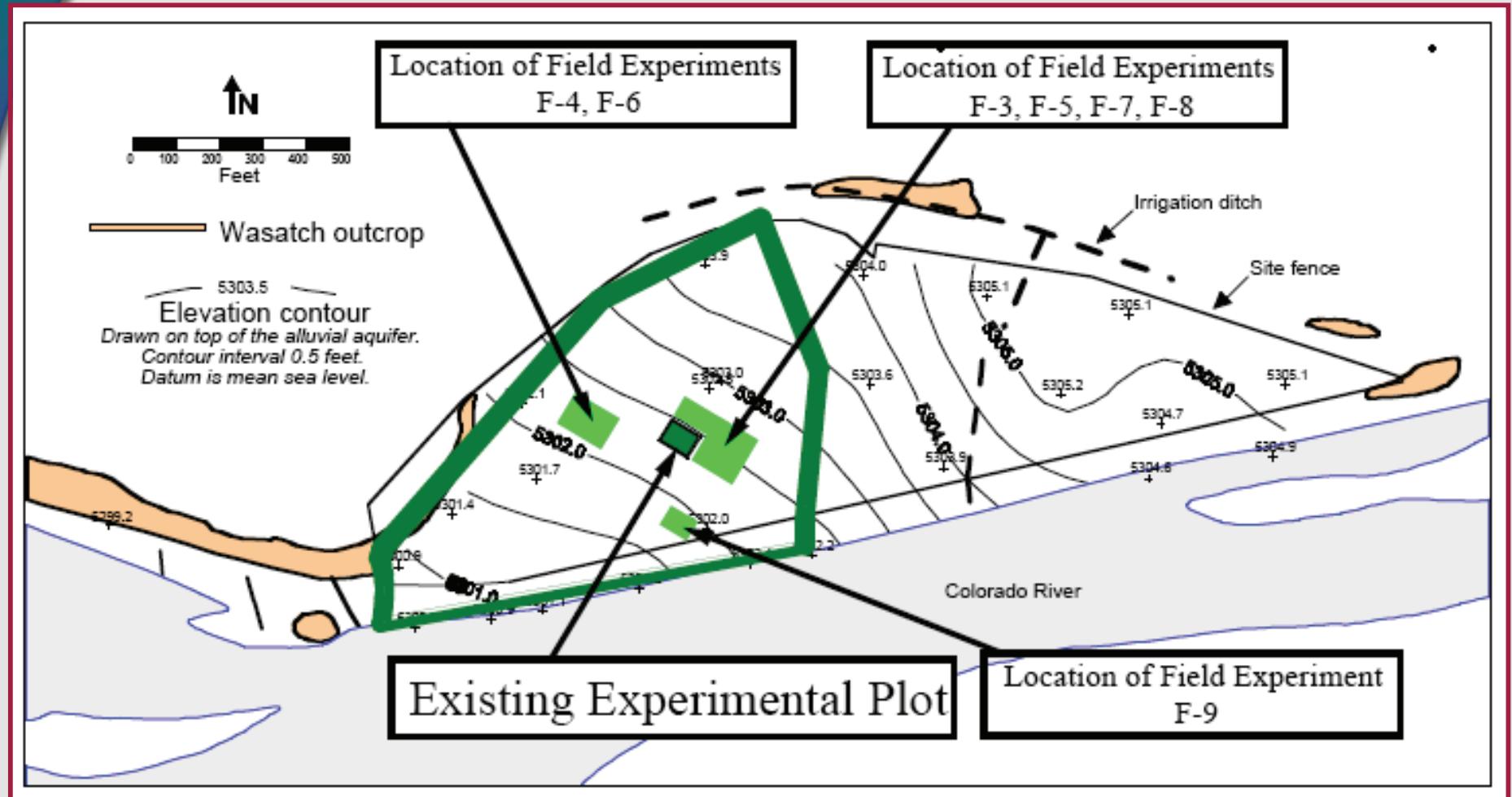
# U(VI) Concentrations (mg/L)



# Overlay of U(VI) Concentrations (mg/L)



# Groundwater flow system

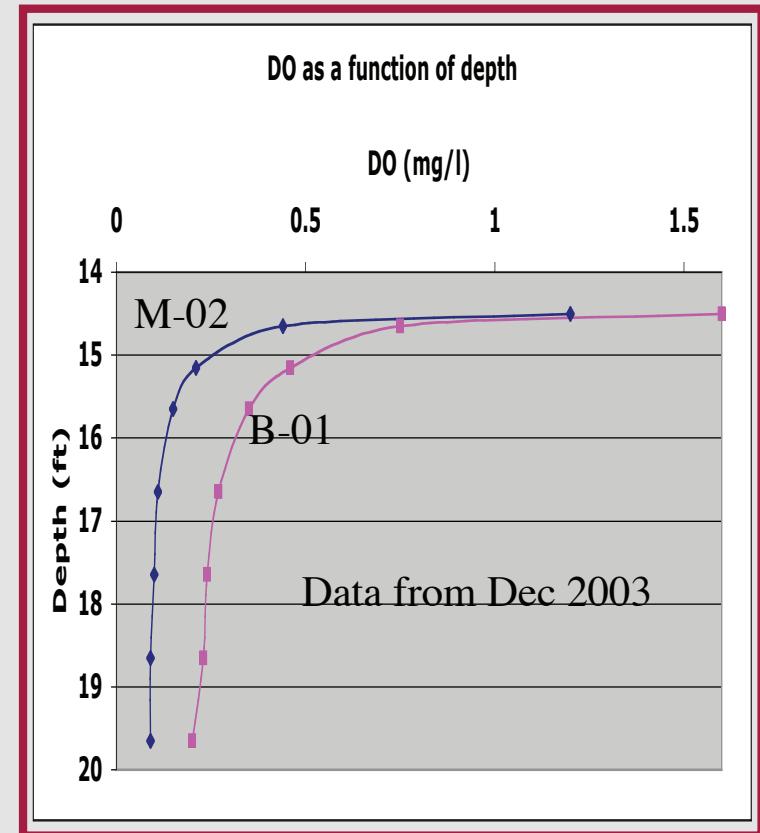


# Baseline Groundwater Geochemistry (U-01 and D-01)

Parameter	Concentration	1 $\sigma$
U(VI) (uM)	0.87	0.07
Conductivity (uS/cm)	2465	42
Temperature (°C)	14.9	1.4
DO (mg/l)	0.29	1.3
Redox (mV)	108	32.6
Alkalinity (meq/l)	8.5	NA
pH	6.97	0.08
Sulfate (mM)	10.98	0.53
Ca (mM)	5.546	NA
Humic DOC (ppm) (well U-02)	2.4	NA
Mn (uM)	21.8	NA
Na (mM)	7.827	NA
Mg (mM)	4.738	NA
K (uM)	218	NA
Cl (mM)	2.76	0.12

# Baseline Geochemical Characteristics (cont.)

Parameter	Concentration
V (uM)	7.19
P (uM)	18.2
Zn (uM)	1.58
As (uM)	0.739
Cu (uM)	0.054
Fe(II) (uM)	21.1 ±6.6
Sulfide (uM)	0.26 ±0.15



# WINCHESTER



## Rifle IFC

- PROTEOMICS
- GENOMICS
- BIOGEOCHEMISTRY
- GEOPHYSICS
- HYDROLOGY

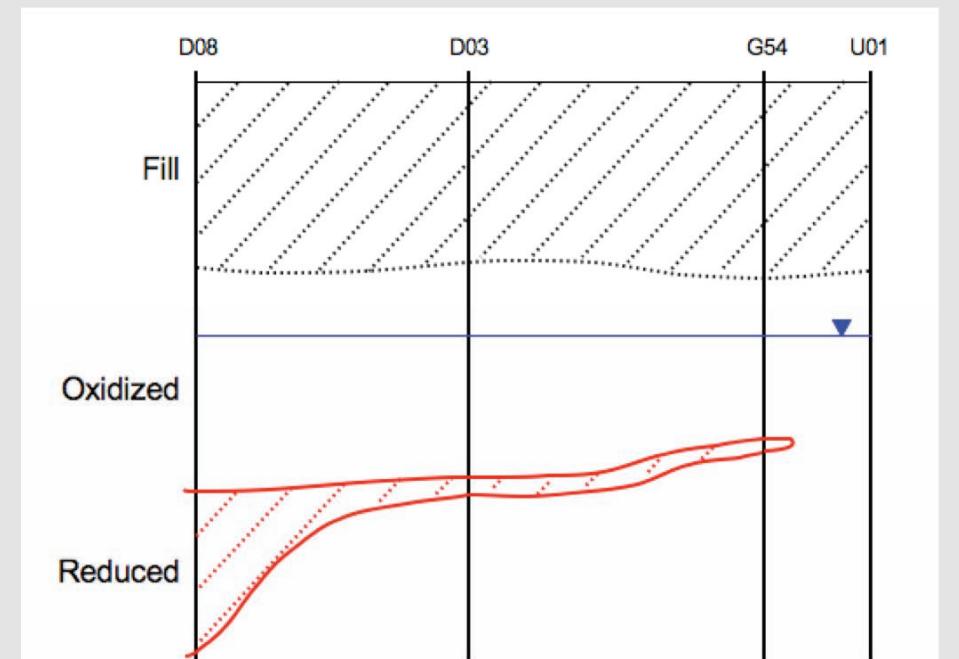
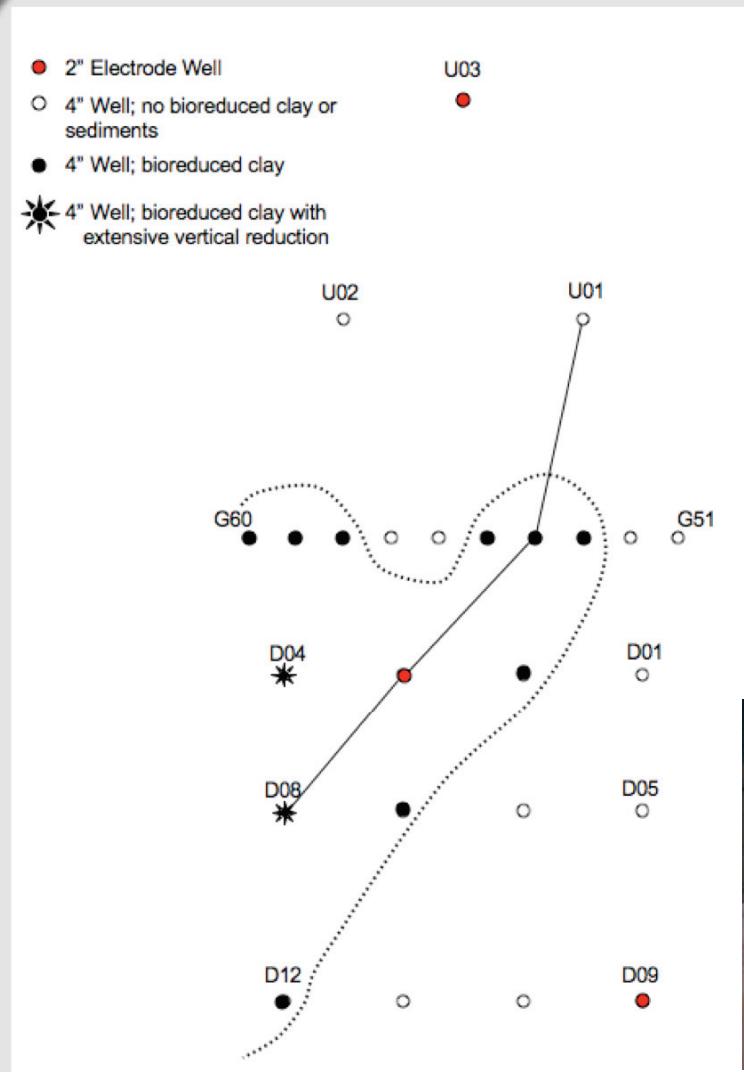
**NO VACANCY**

*Subsurface Science with a Bang!*

**SHOWERS**

- ▶ 2007 field experiment objectives
  - Replicate earlier field experiments showing U(VI) bioreduction by Geobacter to directly link geochemistry, gene expression, proteomics, hydrology, mineralogy, and other data sets
  - Decrease acetate concentration during part of the experiment to evaluate gene expression during acetate limitation
  - Test ability to generate samples for proteomic and metagenomic analysis
- ▶ Unexpected opportunity: access to naturally bioreduced sediment

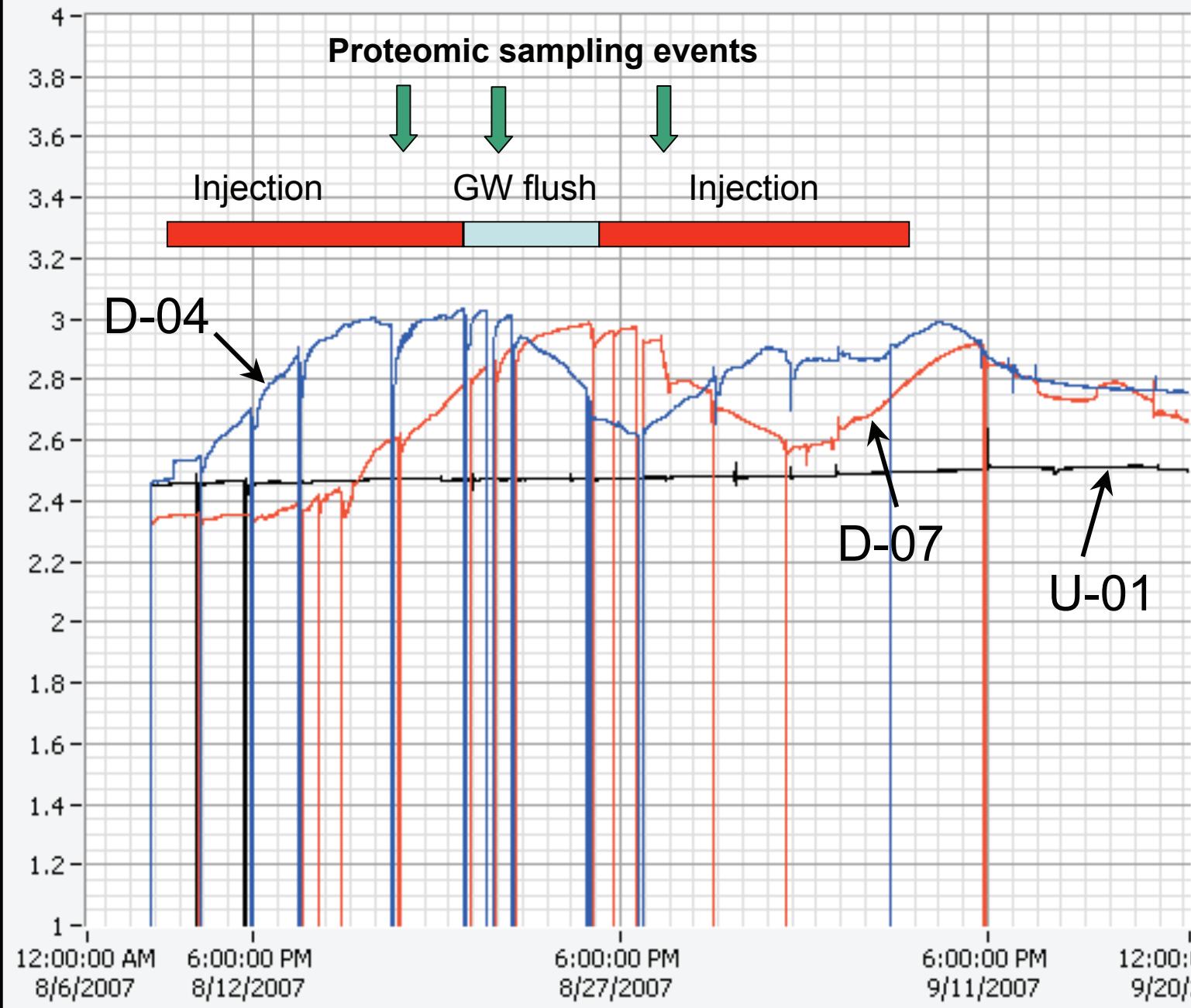
# Winchester Well Layout and Distribution of Reduced Sediments



Rifle\_ERSP\_2: 1. Chemical Data

# RIFLE IFC 2007 "Winchester"

Conductivity (mS/cm)

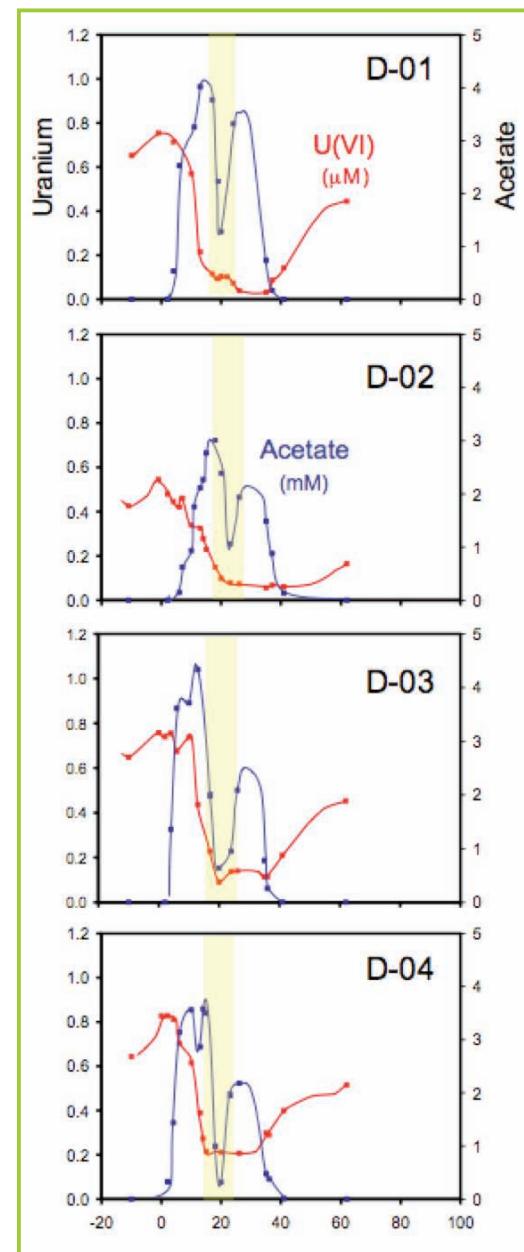




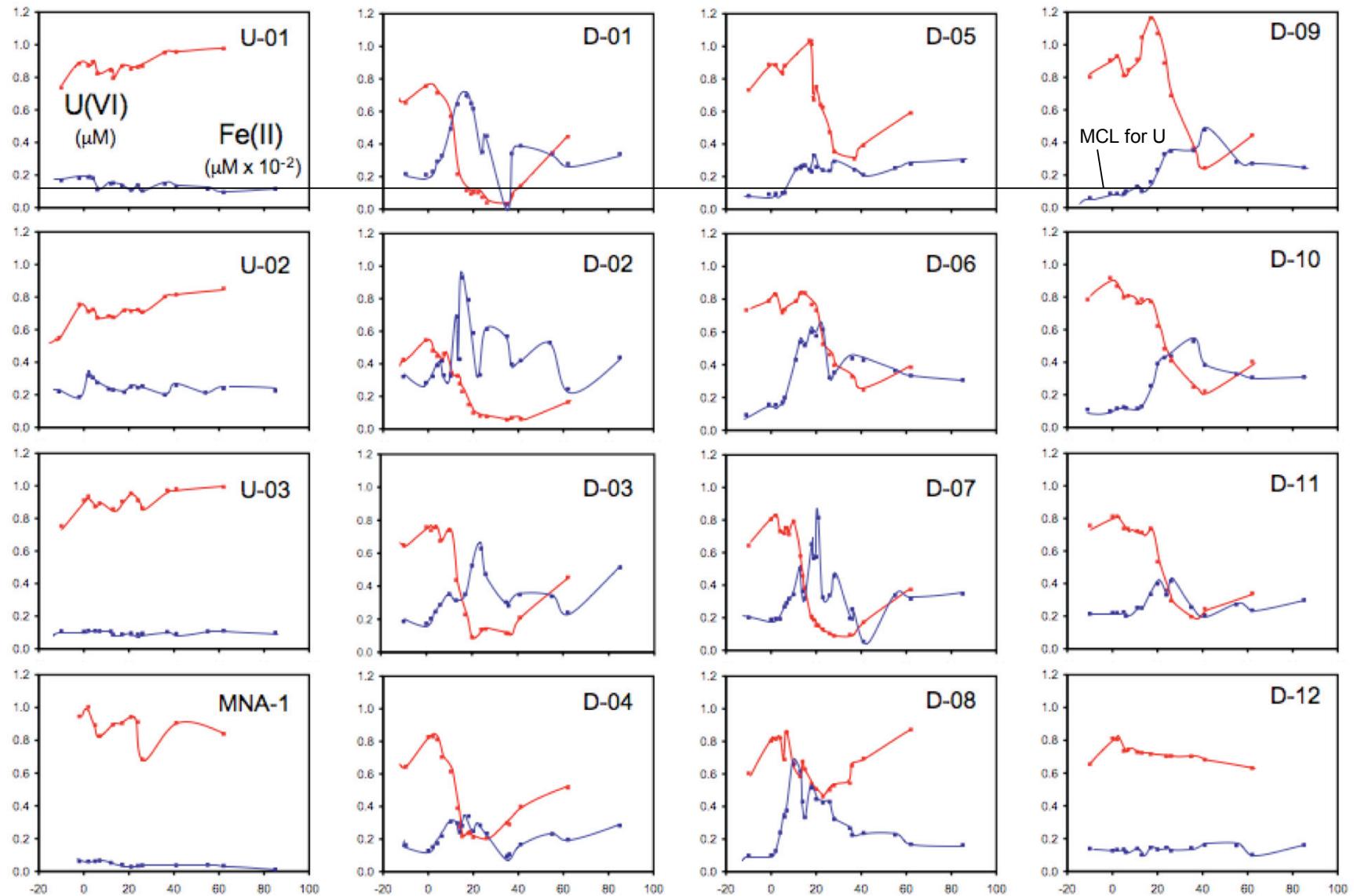
Winchester  
2007  
Well Field and  
Injection gallery



## U and acetate first row wells



# Winchester 2007: Relationship between U(VI) and Fe(II)



N.B. Uranium precipitation even with  $[Ca^{2+}] \approx 3$  to 5 mM

# Winchester Experiment Data Sets

- Groundwater geochemistry (**Poster by Long et al**)
  - Extensive set of geochemical parameters (cations, anions, pH, Eh, T, conductivity, electron donors, electron acceptors, tracer concentrations) collected over time during the course of the experiment
  - Dissolved gas samples suggest the zone of natural bioreduction exerts a strong influence on gas composition both before and after acetate amendment (thanks to help from Brian Spalding).
- Microbiology:
  - Environmental proteomic samples from D-07 and D-05 (**Presentation by Banfield, poster by Wilkins et al.**) provided sufficient material to allow for sequencing and reconstruction of metagenome (work on these samples is underway at JGI).
  - stable isotope probing (**Lee Kerkhof, Poster by Long et al.**)
  - phospholipid fatty acid profiles (Aaron Peacock)
  - temporally extensive set of RNA samples was obtained from numerous downgradient wells linked to nutrients such as ammonium (**Presentation and poster by Lovley et al.**)
- Successful testing of an acetate-sensitive electrode-based sensor (**see poster by Williams et al.**)
- Multi-frequency (0.125, 1, and 4-Hz) induced polarization (IP) data were acquired along two transects oriented perpendicular to groundwater flow at several time points after acetate injection (**see poster by Williams et al.**)
- Hydrologic tests have been conducted before and after biostimulation, enabling us to directly assess the possible impact of biostimulation on the hydraulic properties of the experimental plot (**Frank Spane, poster by Long et al.**)
- A comprehensive set of single borehole (neutron, gamma, and formation conductivity) and cross-borehole (radar) geophysical data was acquired in advance of acetate injection, which when combined with hydrologic testing and tracer data will form the basis of a hydrogeophysical model of the flow cell (**posters by Williams et al. and Hubbard et al.**)
- Mineralogical analyses are underway on sediment samples recovered from the Winchester gallery, with a particular emphasis on the cores transecting the region of natural bioreduction (D05 to D08, **see poster by Campbell et al.**). The naturally bioreduced zone exhibits the highest U and organic carbon concentrations in sediments at the Rifle IFC
- Column experiments guide field experiments and address processes and questions not amenable to field sampling (**see poster by Jaffe et al.**)
- Sorption lab experiments, field experiment upcoming (**Talk on Monday by Davis**)

# 2008 Field Experiments

## ► Big Rusty

- Time course and spatial distribution of gene expression and proteome response
- Transition to sulfate reduction
- Test amendment pulse strategy and manipulation of acetate concentration

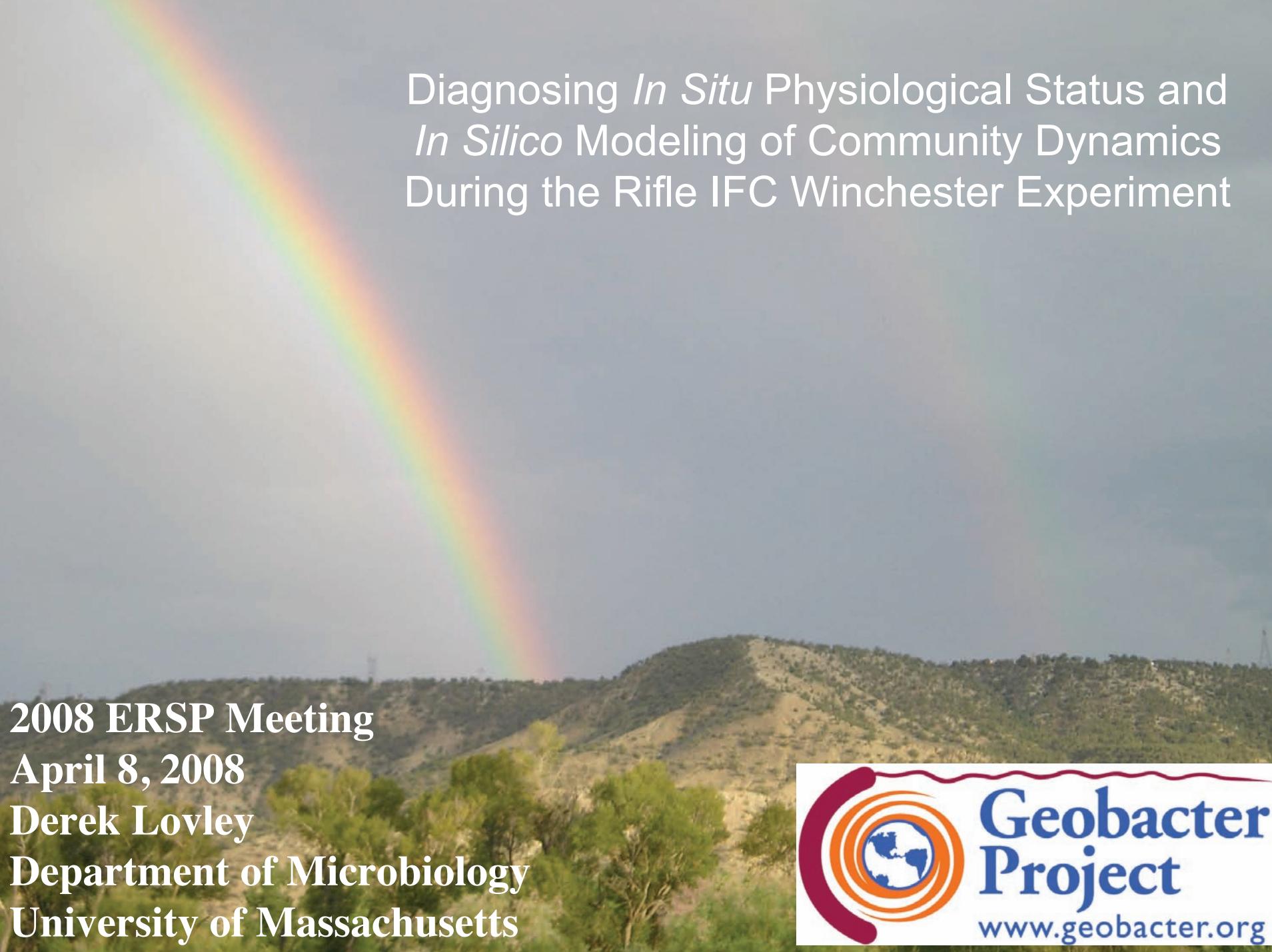
Schedule: July-September

## ► Little Rusty

- Desorption tracer test
- Ambient conditions
- Increase alkalinity
- Precedes an abiotic reductive field experiment

Schedule:

- Well installation, May 19th
- Experiment: September-October



# Diagnosing *In Situ* Physiological Status and *In Silico* Modeling of Community Dynamics During the Rifle IFC Winchester Experiment

2008 ERSP Meeting

April 8, 2008

Derek Lovley

Department of Microbiology

University of Massachusetts



# UMASS Roles in Winchester Experiment

- Monitor composition of microbial community in real time to determine where and when to take samples for metagenomic and proteomic studies
- Diagnose, by quantifying the *in situ* abundance of transcripts for key genes, the *in situ* physiological status of the microorganisms responsible for U(VI) reduction in order to gain insights into the factors controlling the rate and extent of U(VI) reduction
- Further develop the genome-scale *in silico* model of the subsurface community designed to predict the response of the microbial community to various bioremediation strategies prior to implementation in the field

*Geobacter uraniireducens*, Isolated from the Rifle Site and a Member of the Subsurface Clade I  
*Geobacter* that Predominate in a Diversity of Subsurface Environments in Which Dissimilatory Metal Reduction is an Important Process



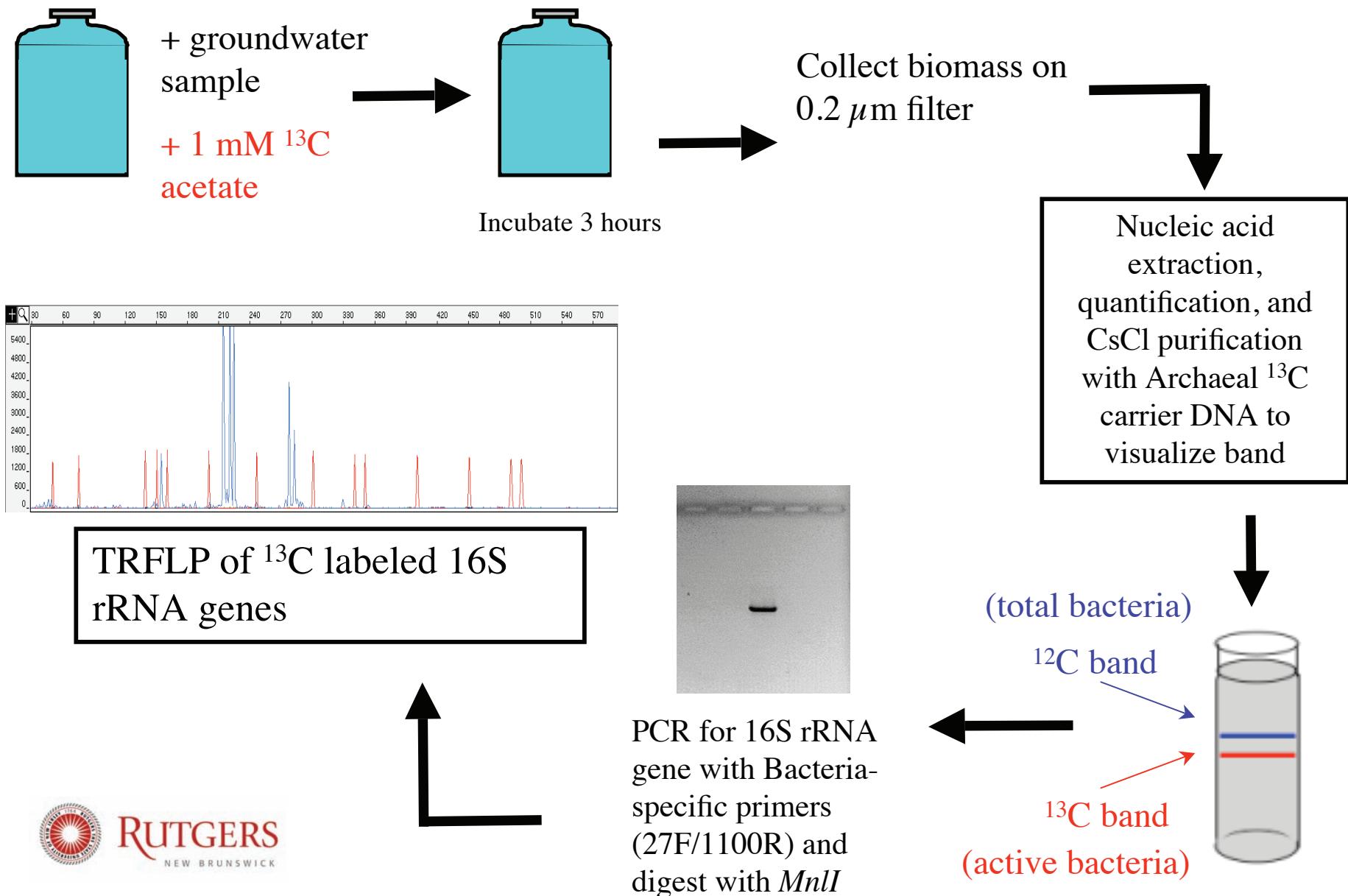
A variety of analyses (16S rRNA sequences; sequences of other conserved genes; lipids; metagenomics; stable isotope probing; proteomics) by a diversity of investigators have demonstrated that *Geobacter* species are the predominant dissimilatory metal-reducing microorganisms during the most effective stages of *in situ* uranium bioremediation at the Rifle IFC site.

*Geobacter uraniireducens*, Isolated from the Rifle Site and a Member of the Subsurface Clade I  
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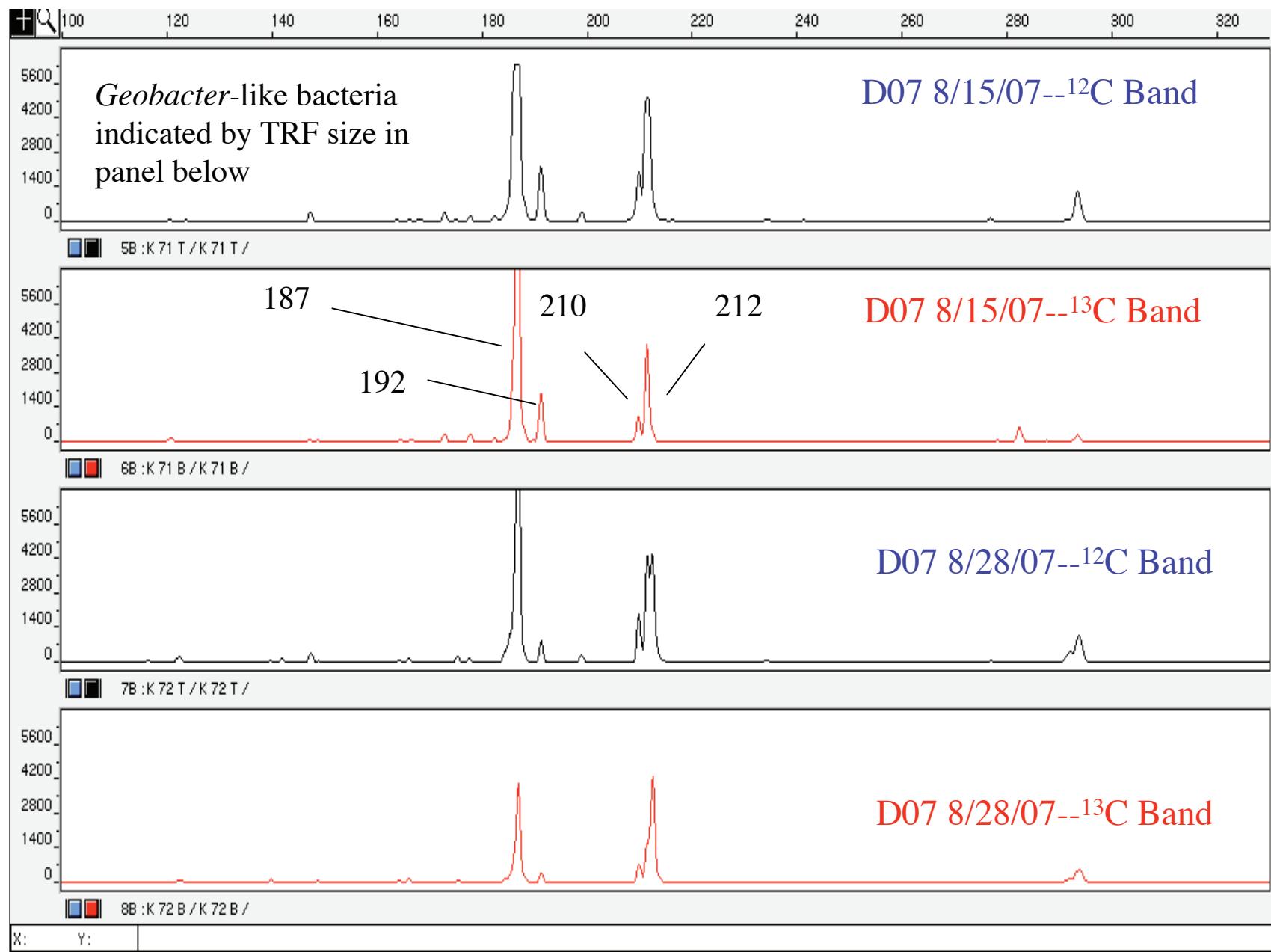


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# Schematic of SIP methodology for Rifle groundwater populations



# Stable Isotope Probing of Groundwater during Winchester Expt.



## Previous Examples of Quantifying Gene Transcript Abundance in the Subsurface to Diagnose *In Situ* Metabolic State During *In Situ* Uranium Bioremediation

### Necessity to Fix Nitrogen

Holmes, D. E., K. P. Nevin, and D. R. Lovley. 2004. *In situ* expression of *Geobacteraceae nifD* in subsurface sediments. Appl. Environ. Microbiol. 70:7251-7259.

### Rates of Metabolism

Holmes, D. E., K. P. Nevin, R. A. O'Neil, J. E. Ward, L. A. Adams, T. L. Woodward, and D. R. Lovley. 2005. Potential for quantifying expression of *Geobacteraceae* citrate synthase gene to assess the activity of *Geobacteraceae* in the subsurface and on current harvesting-electrodes. Appl. Environ. Microbiol. 71:6870-6877.

### Expression of Genes Required for Fe(III) Oxide Reduction

Holmes, D. E., T. Mester, R. A. O'Neil, M. J. Larrahondo, L. A. Adams, R. Glaven, M. L. Sharma, J. A. Ward, K. P. Nevin, and D. R. Lovley. 2008. Genes for two multicopper proteins required for Fe(III) oxide reduction in *Geobacter sulfurreducens* have different expression patterns both in the subsurface and on energy-harvesting electrodes. Microbiology (in press).

### Iron Limitation

O'Neil, R. A., D. E. Holmes, M. V. Coppi, L. A. Adams, M. J. Larrahondo, J. E. Ward, K. P. Nevin, T. L. Woodard, H. A. Vrionis, A. L. N'Guessan, and D. R. Lovley. 2008. Gene transcript analysis of assimilatory iron limitation in *Geobacteraceae* during groundwater bioremediation. Environ. Microbiol.: (in press).

### Oxidative Stress

**Mouser, P. J., D. E. Holmes, L. A. Perpetua, R. DiDonato, B. Postier, A. Liu, and D. R. Lovley.** 2008. Quantifying purative oxidative stress response genes in *Geobacter* species during *in situ* bioremediaiton of uranium-contamianted groundwater: comparision with oxidative stress response in the subsurface isolate *Geobacter uraniireducens*. (manuscript to be submitted)

# Quantifying Gene Transcript Abundance to Diagnose *In Situ* Physiological Metabolic State During *In Situ* Uranium Bioremediation in Winchester (i.e. 2007) Rifle Field Experiment

- Expression of Genes Indicative of Growth Rate
- Expression of Genes Encoding Acetate Transporters
- Expression of Genes Involved in Phosphate Uptake
- Expression of Genes Involved in Uptake of Ammonium and Nitrogen Fixation

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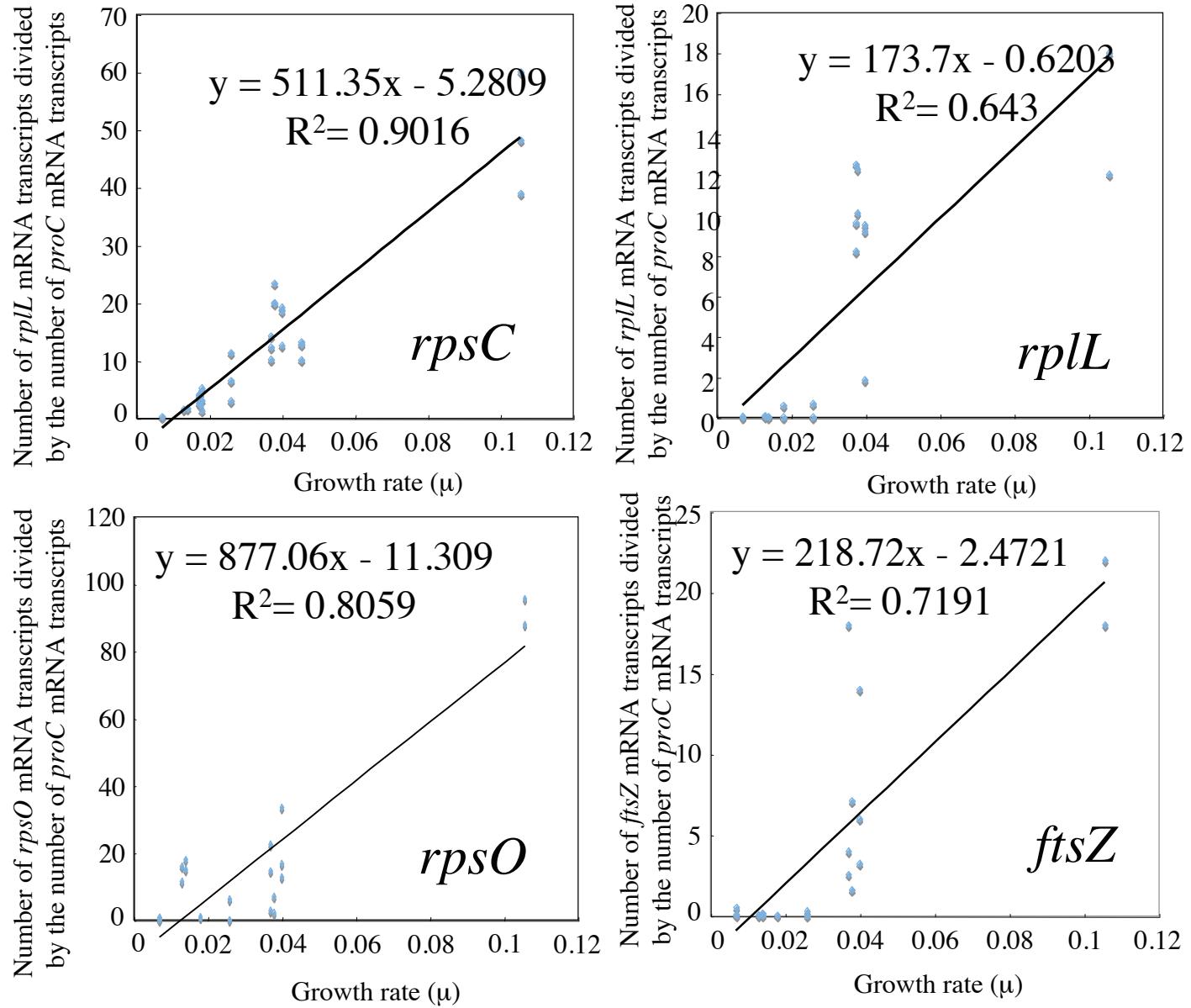
- Expression of Genes Indicative of Growth Rate - Dawn Holmes
- Expression of Genes Encoding Acetate Transporters
- Expression of Genes Involved in Phosphate Uptake
- Expression of Genes Involved in Uptake of Ammonium and Nitrogen Fixation

Three Microarray Studies Identified 4 Genes in  
*Geobacter uraniireducens* that were Indicative of  
Growth Rate: *rpsC*, *rplL*, *rpsO*, and *ftsZ*

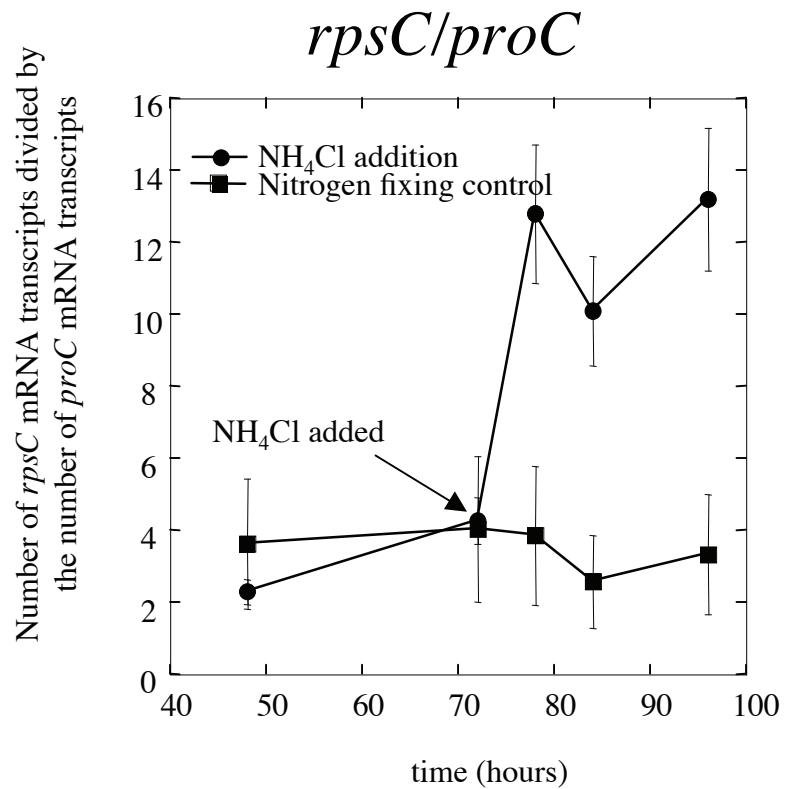
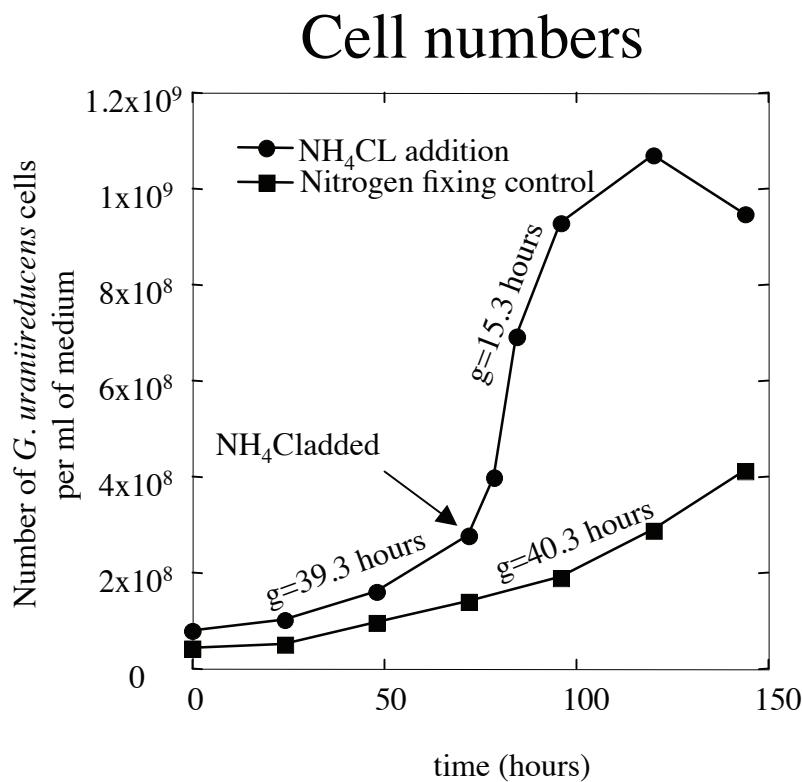
Gene name	Gene annotation	Locus ID	Fold change sediment vs fumarate	Fold change Fe(III) oxide vs fumarate	Fold change Mn(IV) oxide vs fumarate
<i>rpsC</i>	ribosomal protein S3	Gura_1073	19.33	2.87	6.40
<i>rplL</i>	ribosomal protein L7/L12	Gura_1060	20.13	3.21	5.71
<i>rpsO</i>	ribosomal protein S15	Gura_1905	13.81	2.12	5.89
<i>ftsZ</i>	cell division protein FtsZ	Gura_3968	3.41	1.49	1.83

Holmes et al. (in prep.)

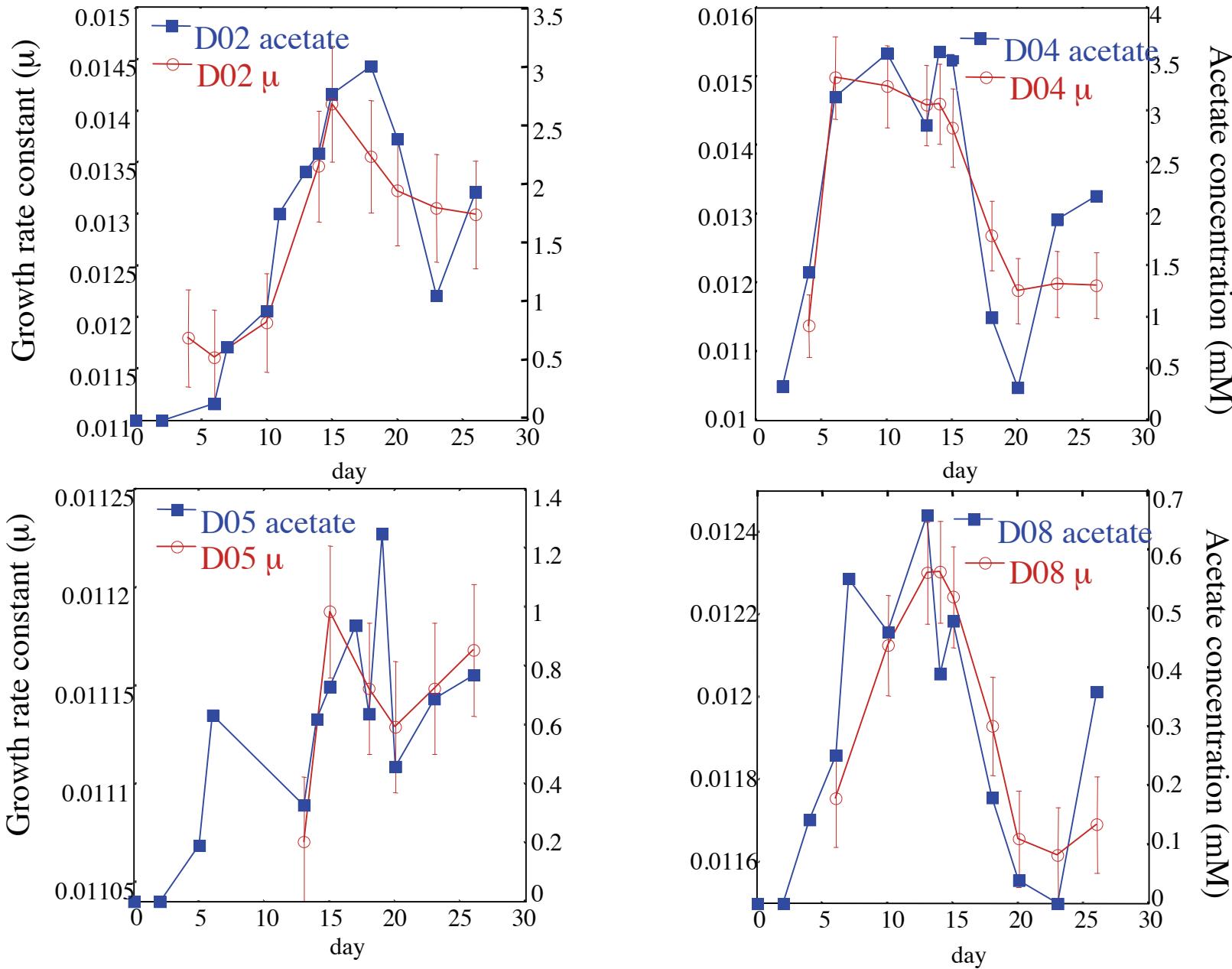
# Expression of the Gene Encoding the Ribosomal protein, *RpsC*, Correlated Most Strongly with *G. uraniireducens* Growth Rate



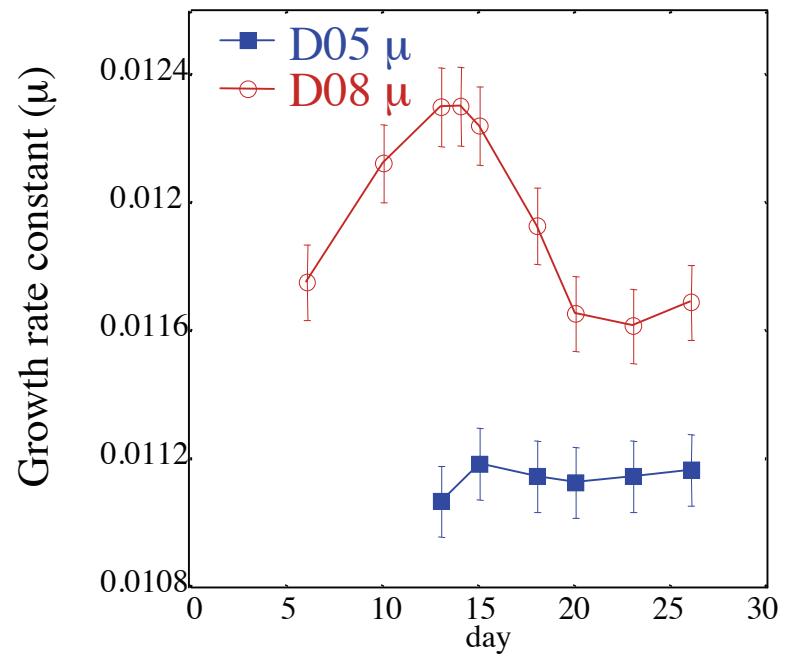
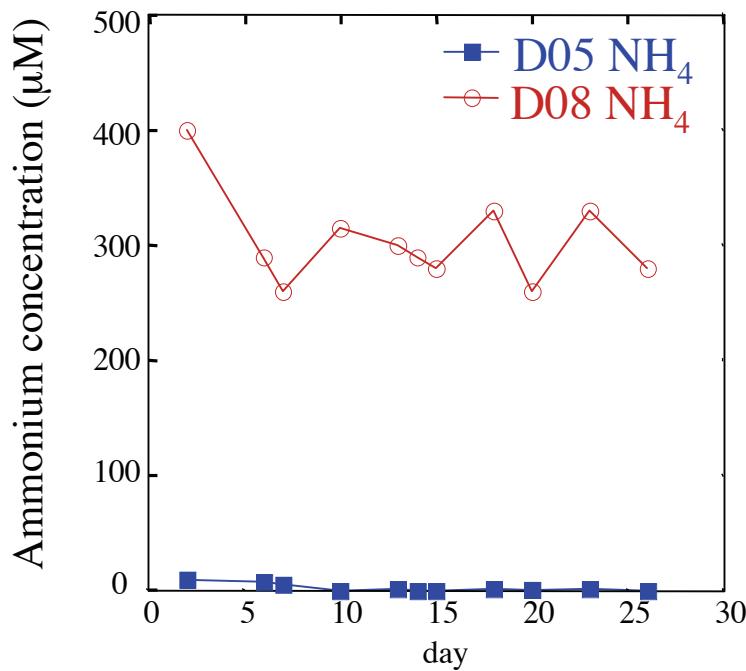
Transcript Abundance of *rpsC* (in comparison to abundance of the transcripts for the house-keeping gene, *proC*) Changes Rapidly In Response to Changes in Growth Rate, such as when Ammonium is Added to a Nitrogen-Fixing Culture



# *In Situ* Growth Rates of *Geobacter* species, Estimated by Quantifying *rpsC* Transcript Abundance, Track Well With Acetate Availability



Estimated Growth Rates Were Higher in Well D08, Which had High Ammonium Concentrations (ca. 300  $\mu\text{M}$ ) than in Well D05, which had Low Ammonium Concentrations

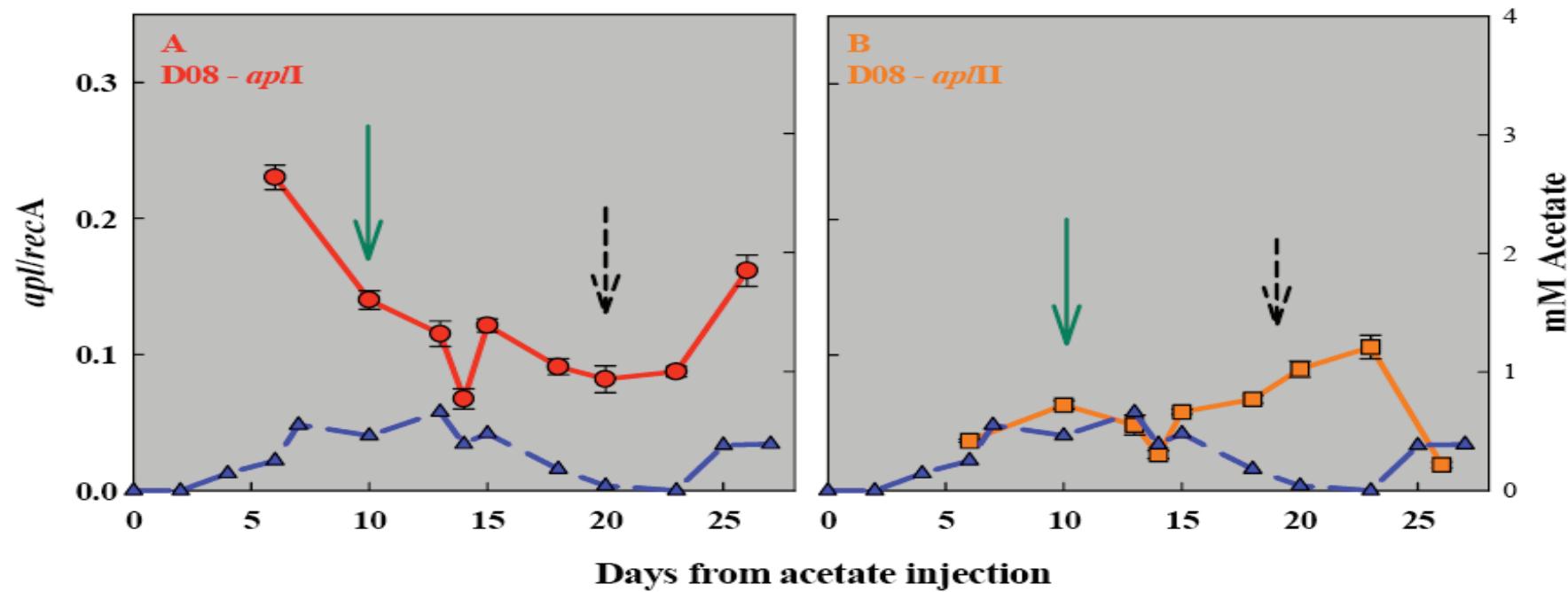


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- Expression of Genes Indicative of Growth Rate
- Expression of Genes Encoding Acetate Transporters - Hila Elifantz
- Expression of Genes Involved in Phosphate Uptake
- Expression of Genes Involved in Uptake of Ammonium and Nitrogen Fixation

Pure Culture Studies Identified Two Classes of Acetate Transporter Genes, *aplI* and *aplII*, that are Highly Conserved in *Geobacter* species and are Upregulated Under Acetate-Limiting Conditions in Chemostats

- Abundance of *aplI* and *aplII* transcripts increased with decreases in acetate availability at D-08 where relatively high concentrations of ammonium were available

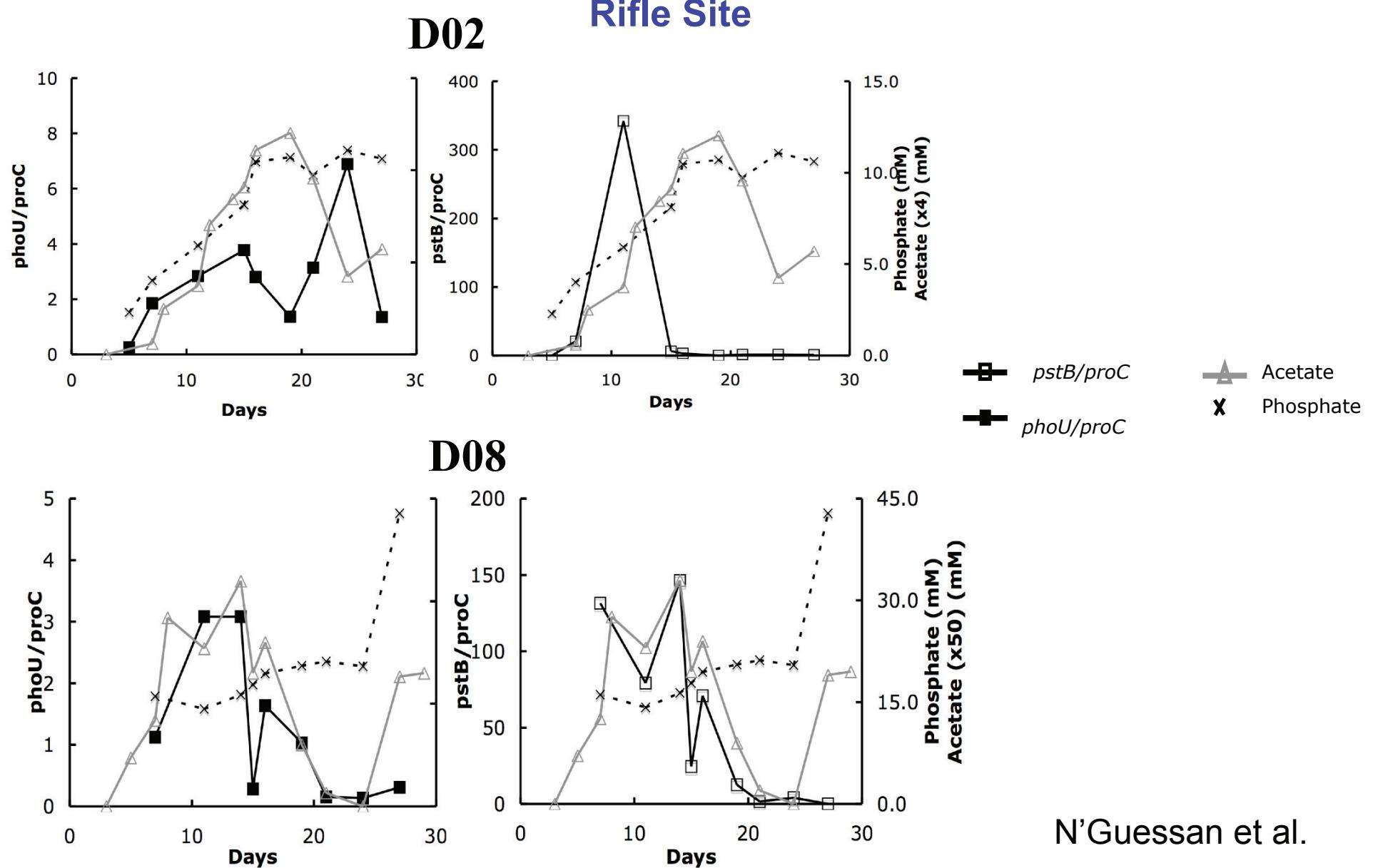


- Transcript abundance patterns at other sites were more complicated, possibly due to limitations in the availability of ammonium

# Quantifying Gene Transcript Abundance to Diagnose *In Situ* Physiological Metabolic State During *In Situ* Uranium Bioremediation in Winchester (i.e. 2007) Rifle Field Experiment

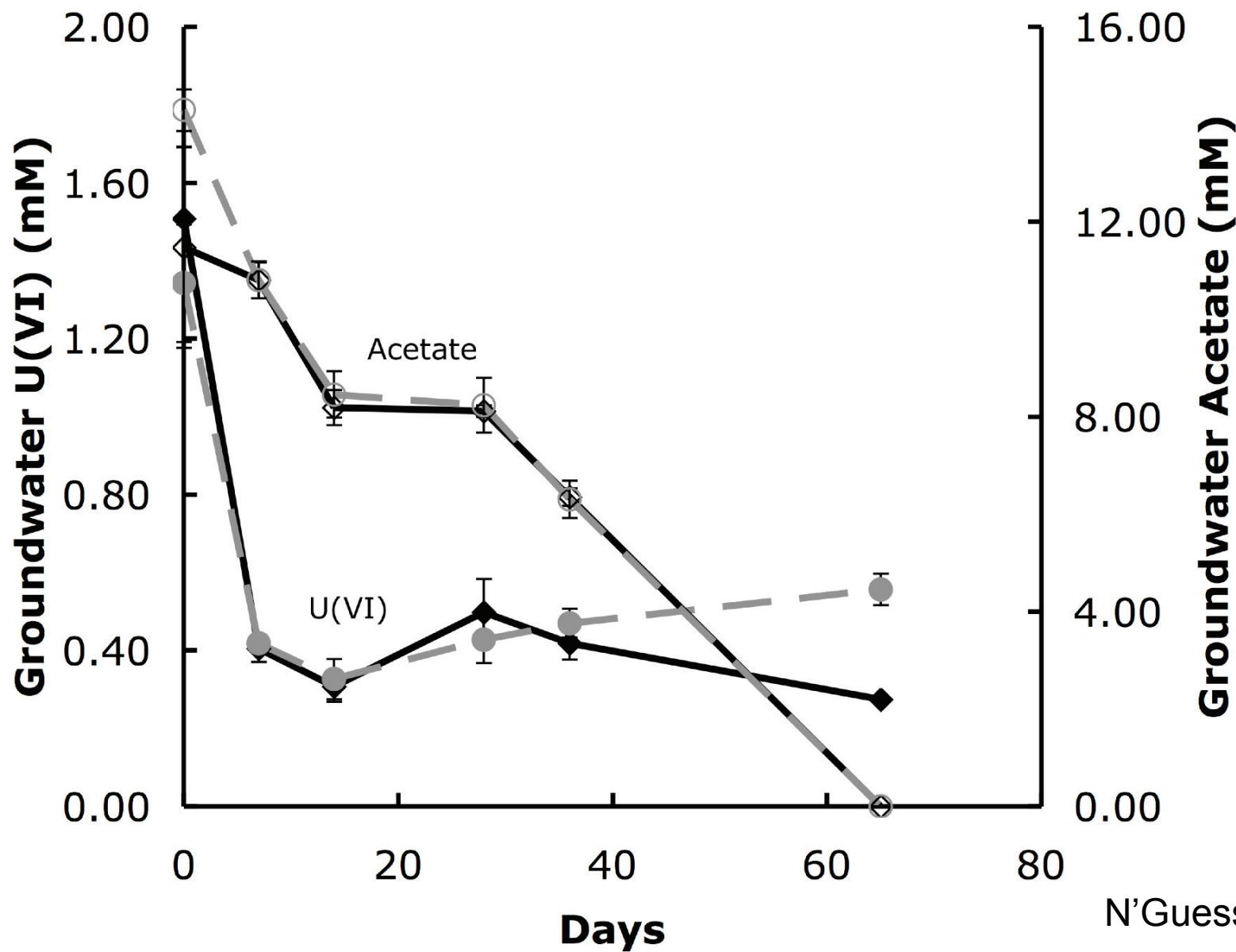
- Expression of Genes Indicative of Growth Rate
- Expression of Genes Encoding Acetate Transporters
- Expression of Genes Involved in Phosphate Uptake - Lucie N'Guessan
- Expression of Genes Involved in Uptake of Ammonium and Nitrogen Fixation

The Presence of Phosphate in the Groundwater and the Expression Patterns of *pstB* and *phoU* Suggest that the Growth of *Geobacter* species is not Phosphate Limited During *In Situ* Uranium Bioremediation at the Rifle Site



N'Guessan et al.

# Amendment of inorganic phosphate to batch incubations did not improve U(VI) removal

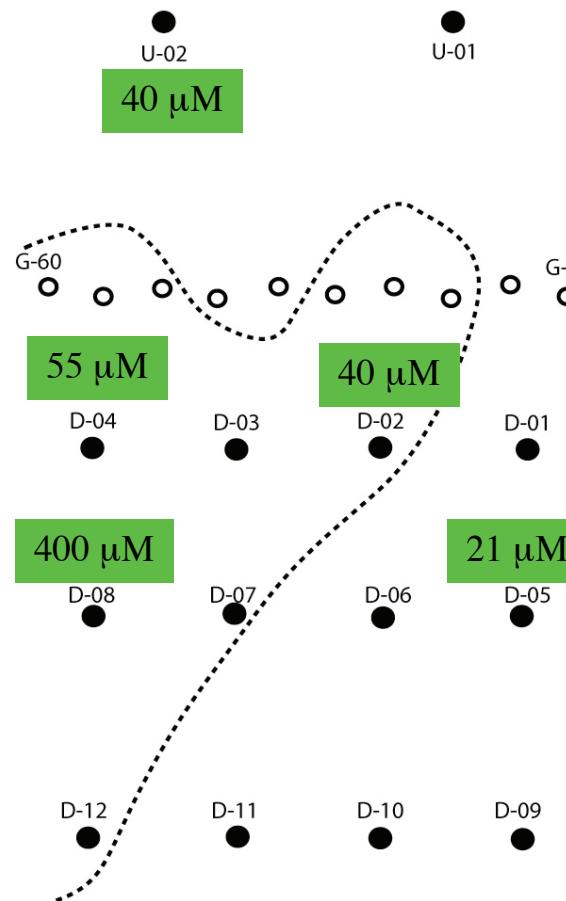


N'Guessan et al.

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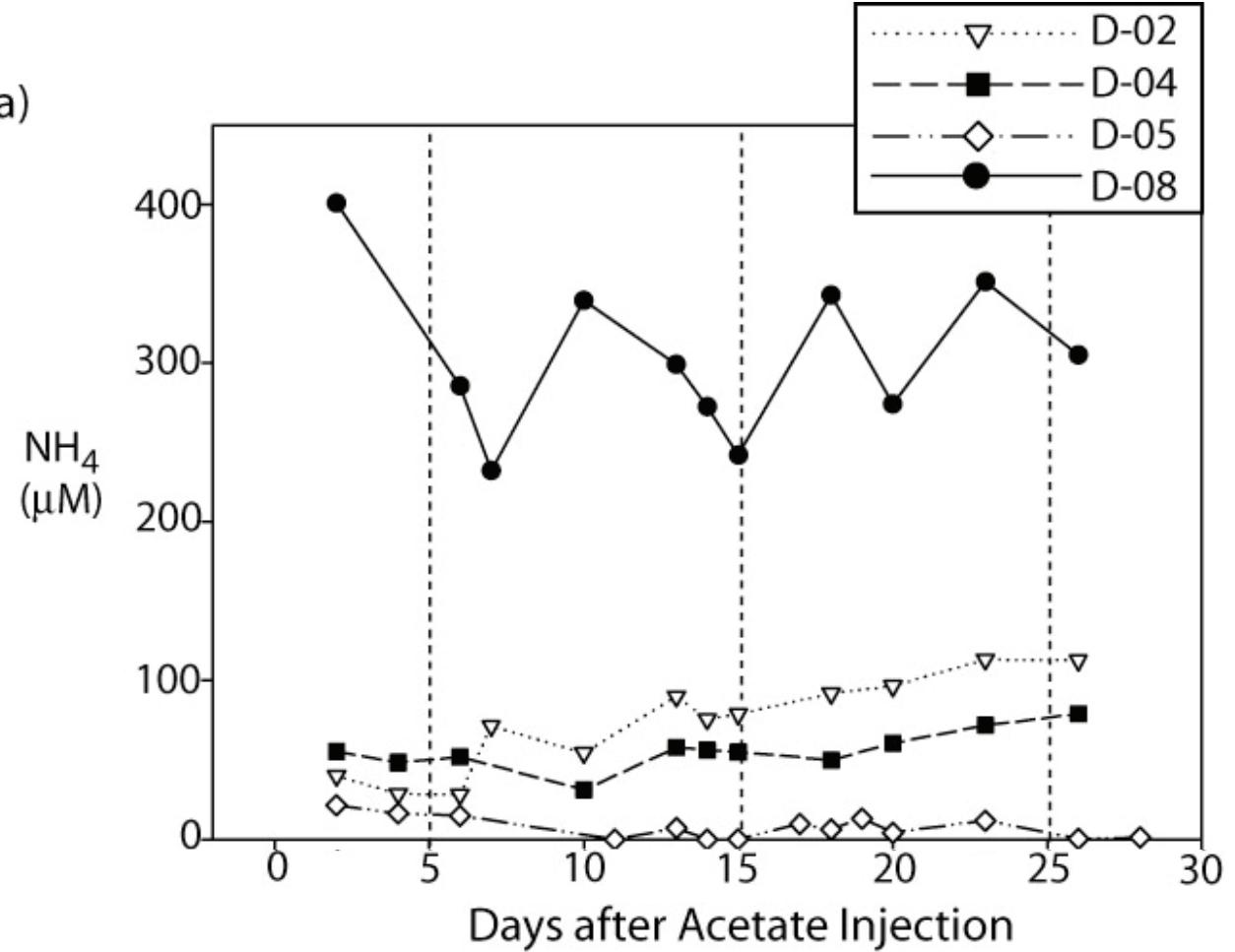
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Paula Mouser
-

# The Availability of Ammonia Varied with the Treatment Zone at the Rifle Site in 2007



Initial  $\text{NH}_4$   
Concentrations

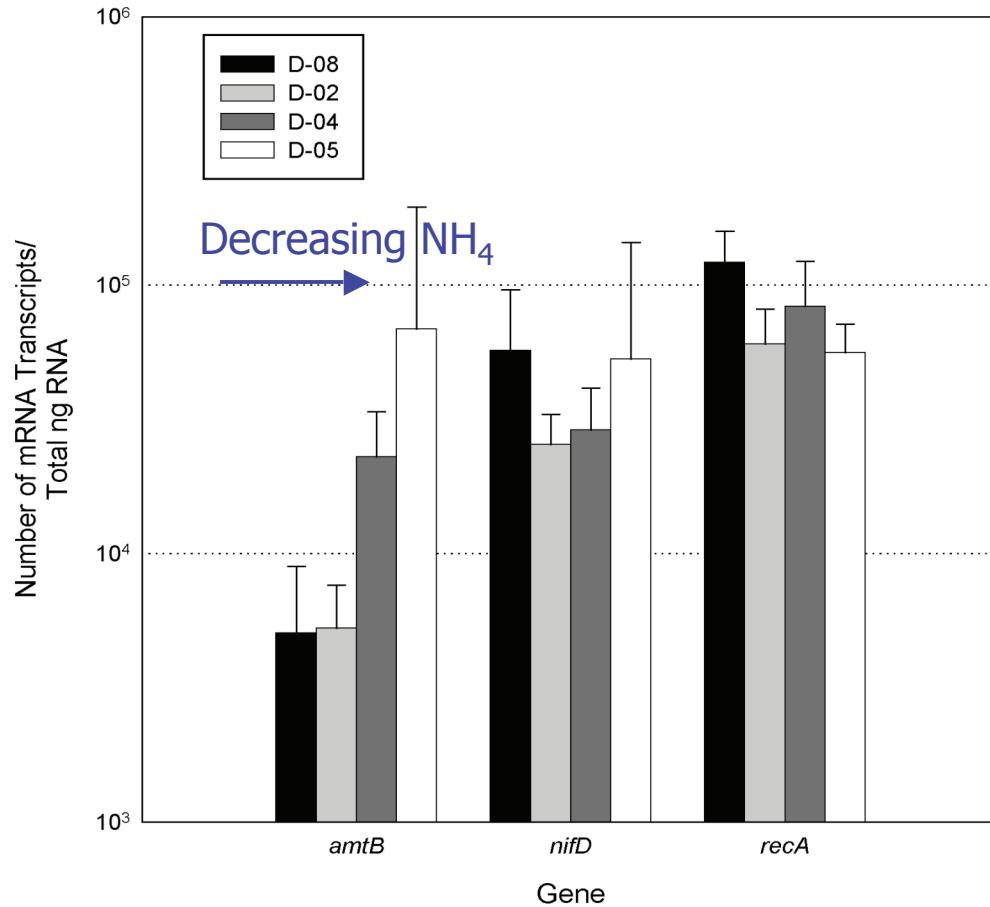
a)



$\text{NH}_4$  trends in amended wells

Mouser et al. (in preparation)

# Transcript Abundance of Genes for the Ammonium Transporter, *amtB*, the Nitrogen-Fixation Gene, *nifD*, and the House-Keeping Gene, *recA* During *In Situ* Uranium Bioremediation at Four Zones at the Rifle Site



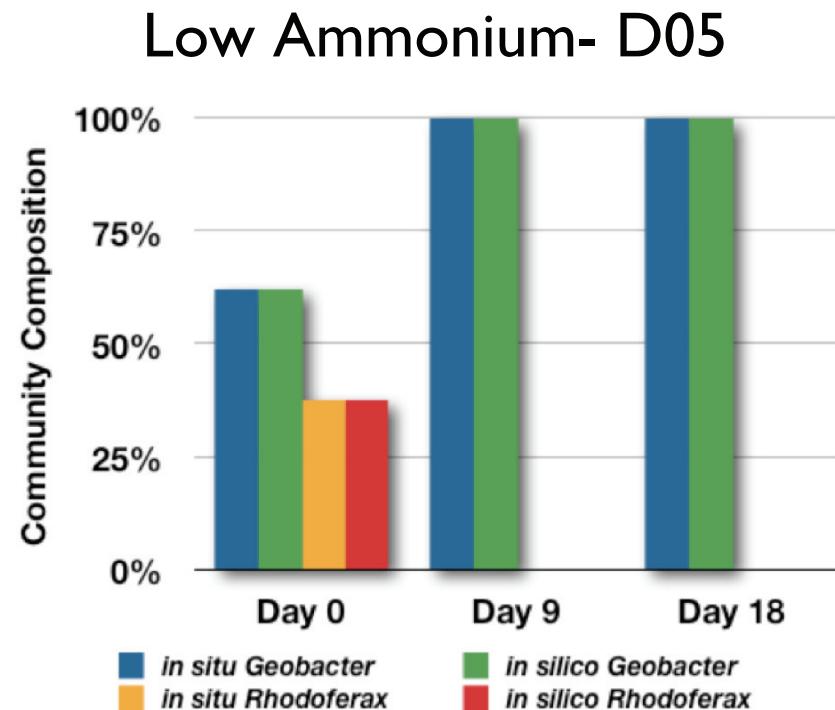
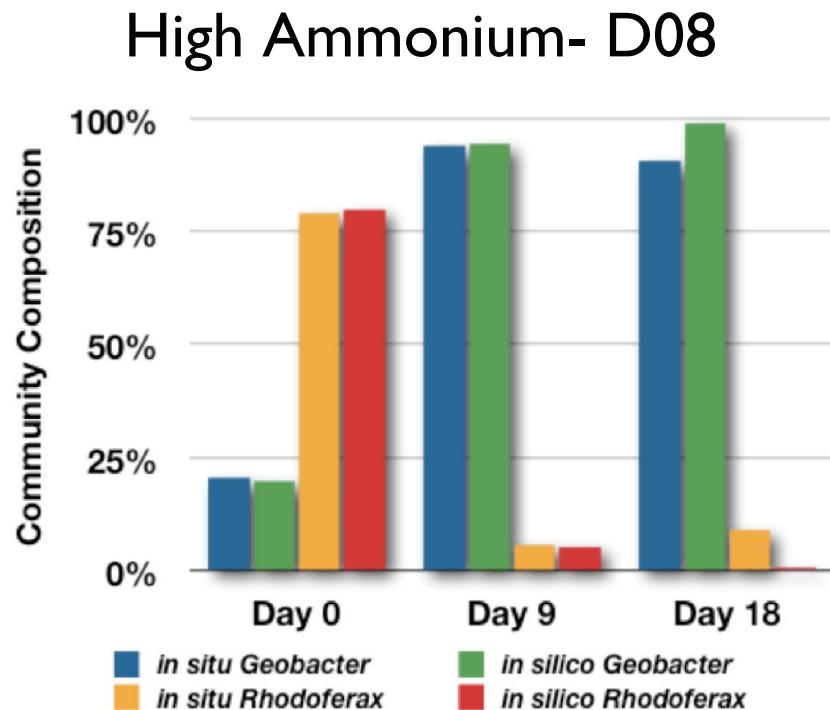
- The abundance of transcripts for *amtB*, increased with decreasing ammonium availability
- With the exception of one day at D-05 when ammonia concentrations dropped to zero, *nifD*, was expressed at constitutive levels at all sites throughout the treatment period, suggesting that, with that one exception, *Geobacter* species were utilizing ammonia

## Ammonia Availability Appears to Influence the Composition of Microbial Community Naturally Present at the Rifle Site

- The activity of metal-reducing microorganisms prior to acetate amendment at the Rifle site is of interest in understanding the natural attenuation via U(VI) reduction taking place at the site
- *Rhodoferax* and *Geobacter* species are both acetate-oxidizing Fe(III) reducers
- At D-08, the high-ammonium site, *Rhodoferax* species are 4-fold more abundant than *Geobacter* species
- At low-ammonium sites *Rhodoferax* and *Geobacter* species are of comparable abundance
- With the addition of acetate to the subsurface *Geobacter* species outcompete *Rhodoferax* and becomes the dominant organisms at all sites

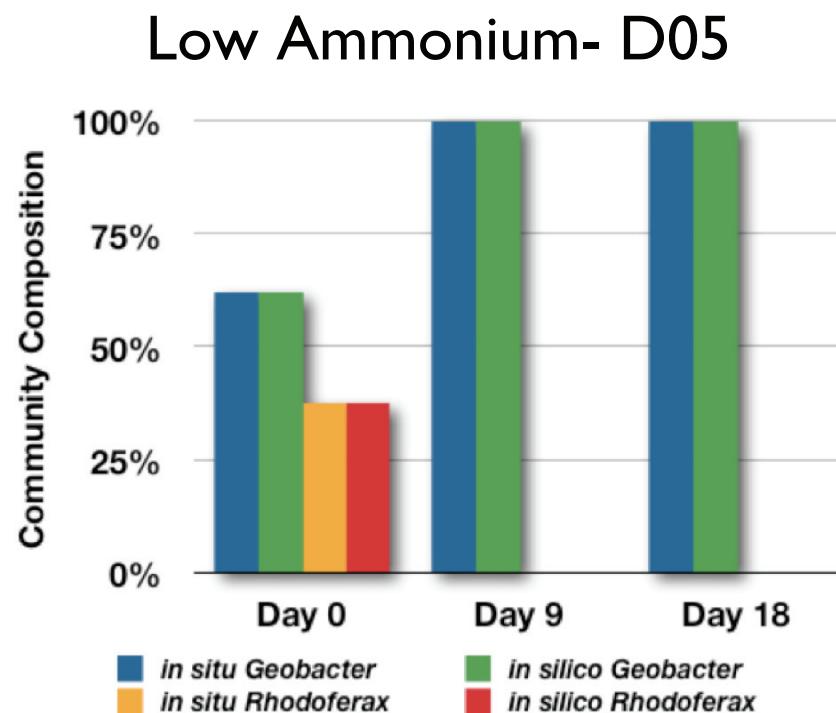
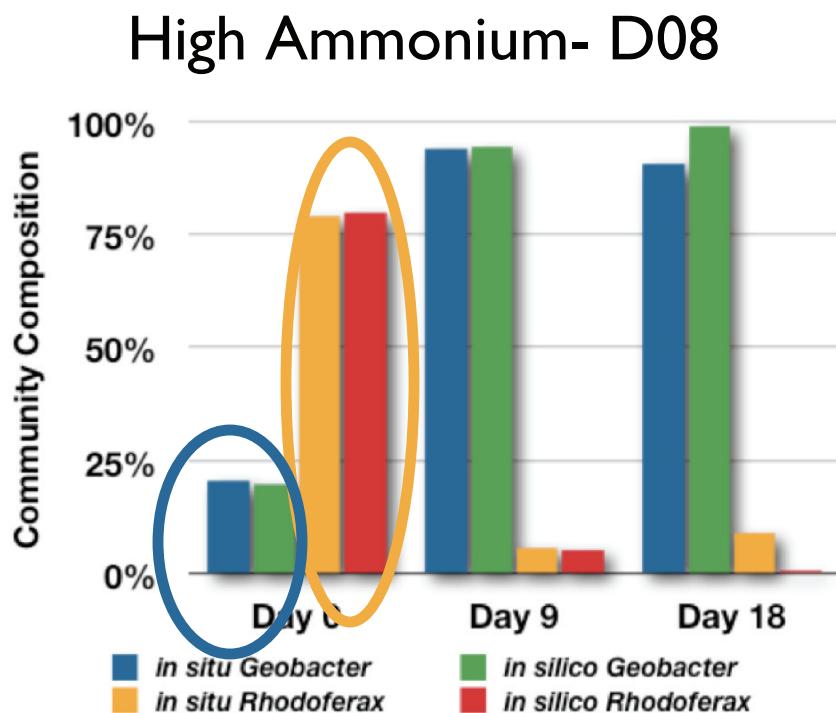
# Modeling Community Dynamics During *In Situ* Uranium Bioremediation

- *In silico* models of *Geobacter* and *Rhodoferax* utilized physiological parameters determined in representative pure cultures
- Relative proportions of organisms determined prior to addition of acetate and at time points following the addition of acetate
- *In silico* predictions match well with field observations



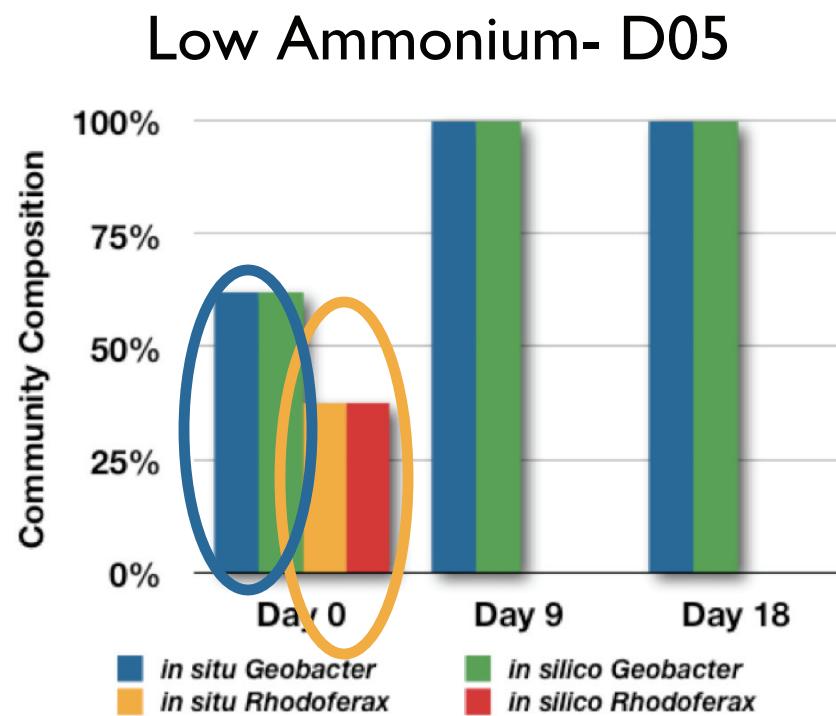
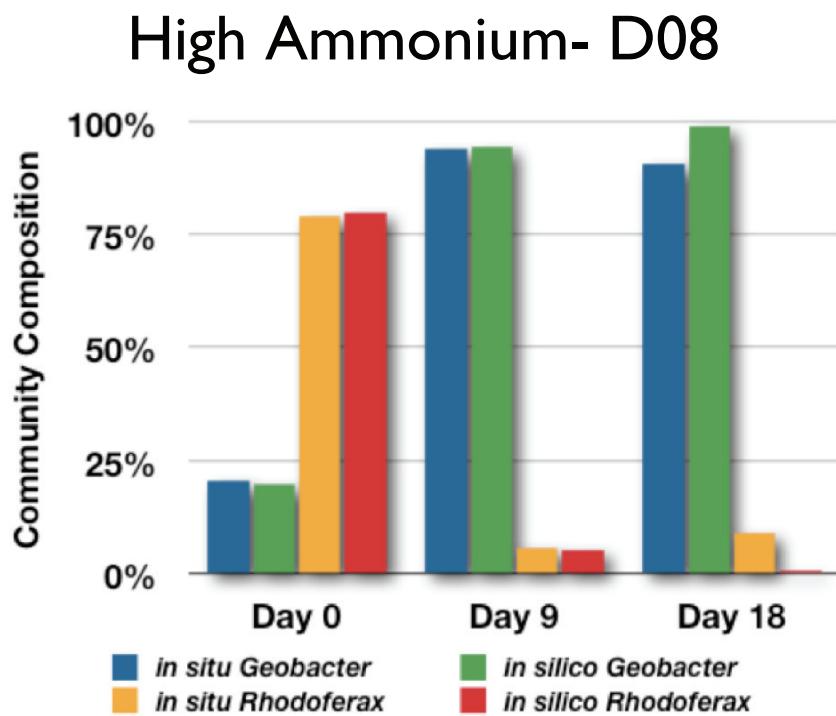
# Modeling Community Dynamics During *In Situ* Uranium Bioremediation

- Prior to Acetate Additions *Rhodoferax* predominated over *Geobacter* at the high ammonium site whereas their abundance was comparable at the low ammonium site



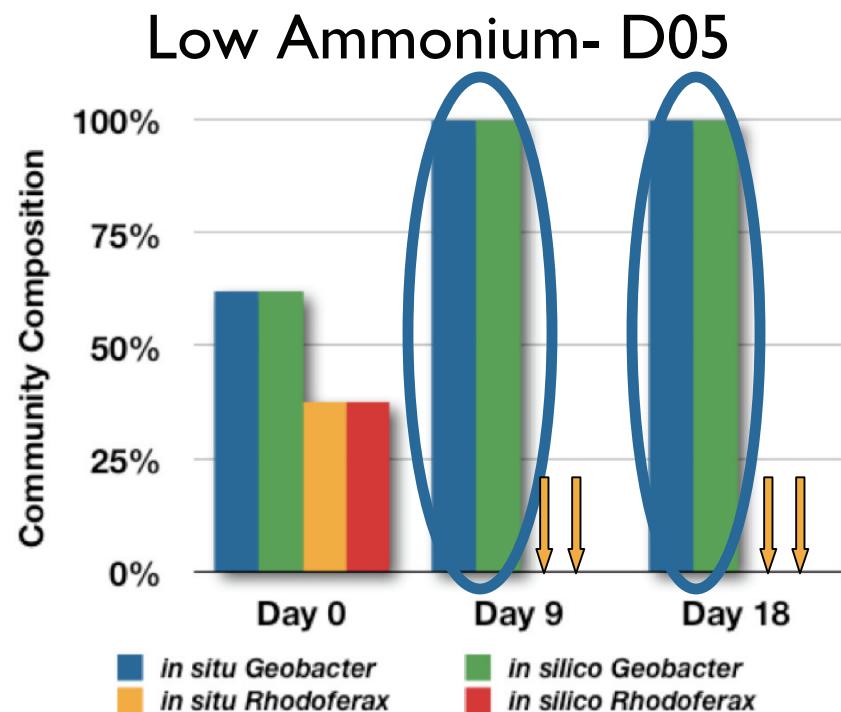
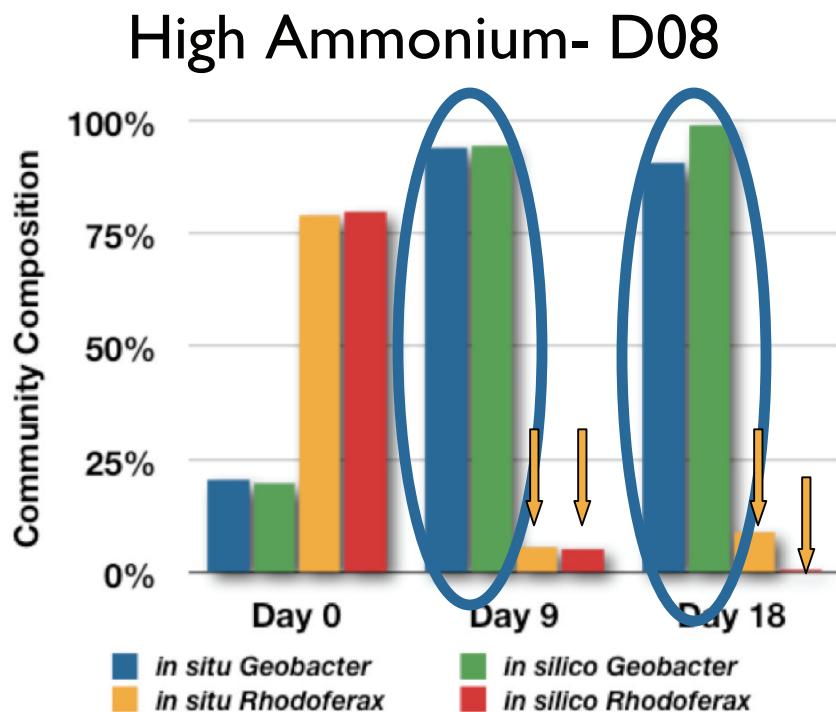
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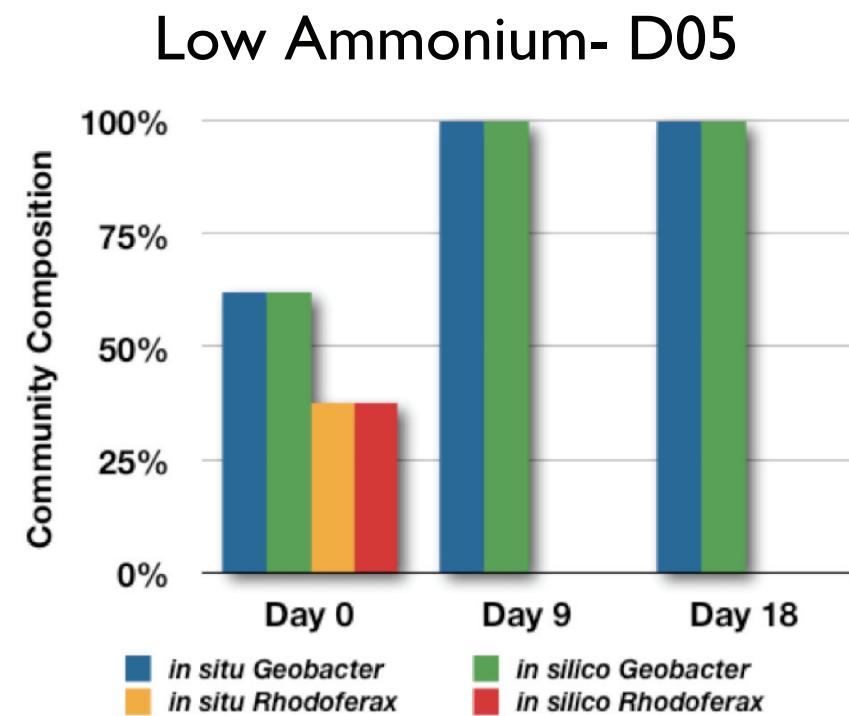
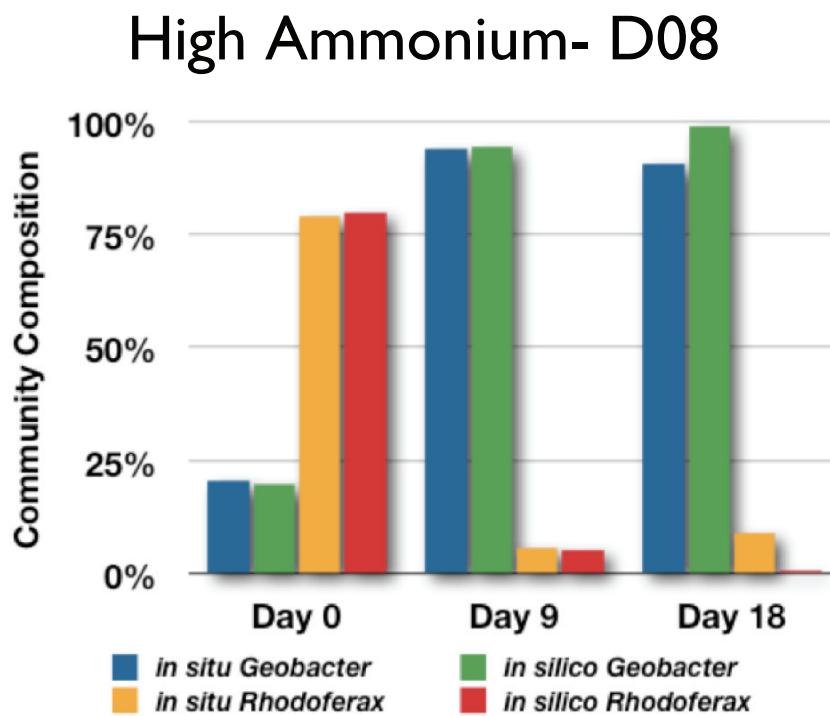
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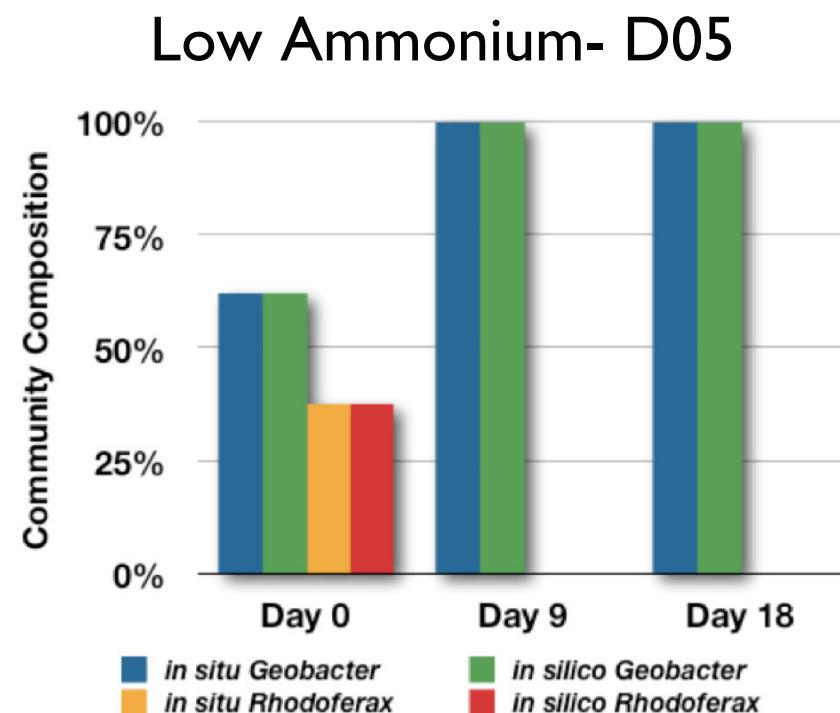
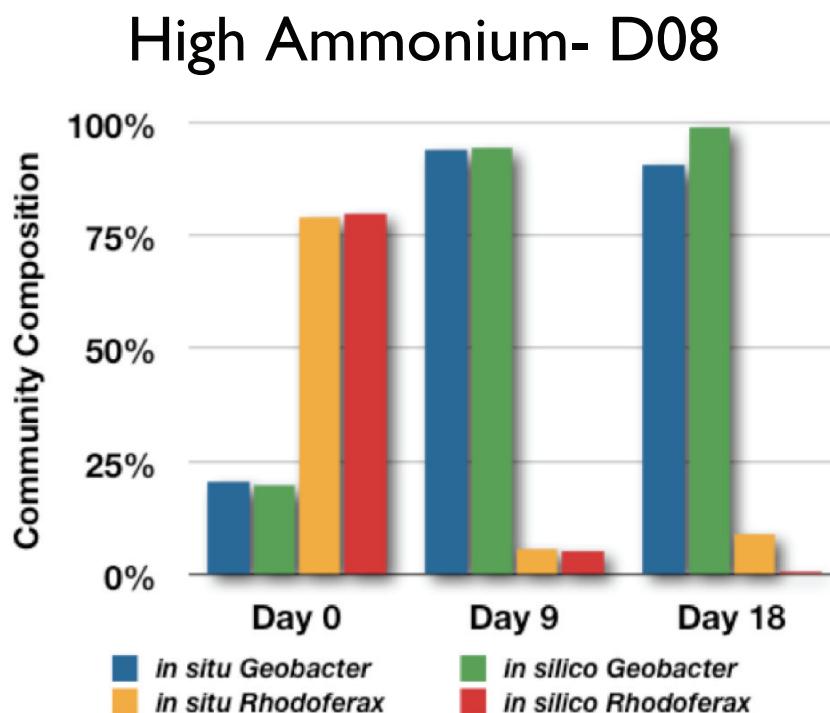
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# Modeling Community Dynamics During *In Situ* Uranium Bioremediation

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- *In silico* modeling predicts that *Geobacter* species will outcompete *Rhodoferax* species when acetate concentrations are high because of faster growth rates
- *Geobacter* species also has the advantage over *Rhodoferax* species under limiting ammonium conditions because it can fix nitrogen whereas *Rhodoferax* cannot



## Plans for 2008 Field Experiment

1. Further evaluation of electron donor and nutrient availability via quantification of gene transcript abundance
2. Evaluate metabolic rates of *Geobacter* species by quantifying *in situ* levels of key enzymes with specific antibodies
3. Obtain additional metagenomic sequences of Rifle site
4. Evaluate the possibility of tracking sulfate reducer activity by quantifying transcript abundance of *dsrA*
5. Isolate environmentally relevant sulfate reducers and sequence their genomes to provide data for *in silico* modeling of competition between *Geobacter* species and sulfate reducers
6. Obtain quantitative microbiological and geochemical data required for next level of genome-based *in silico* modeling of the Rifle site

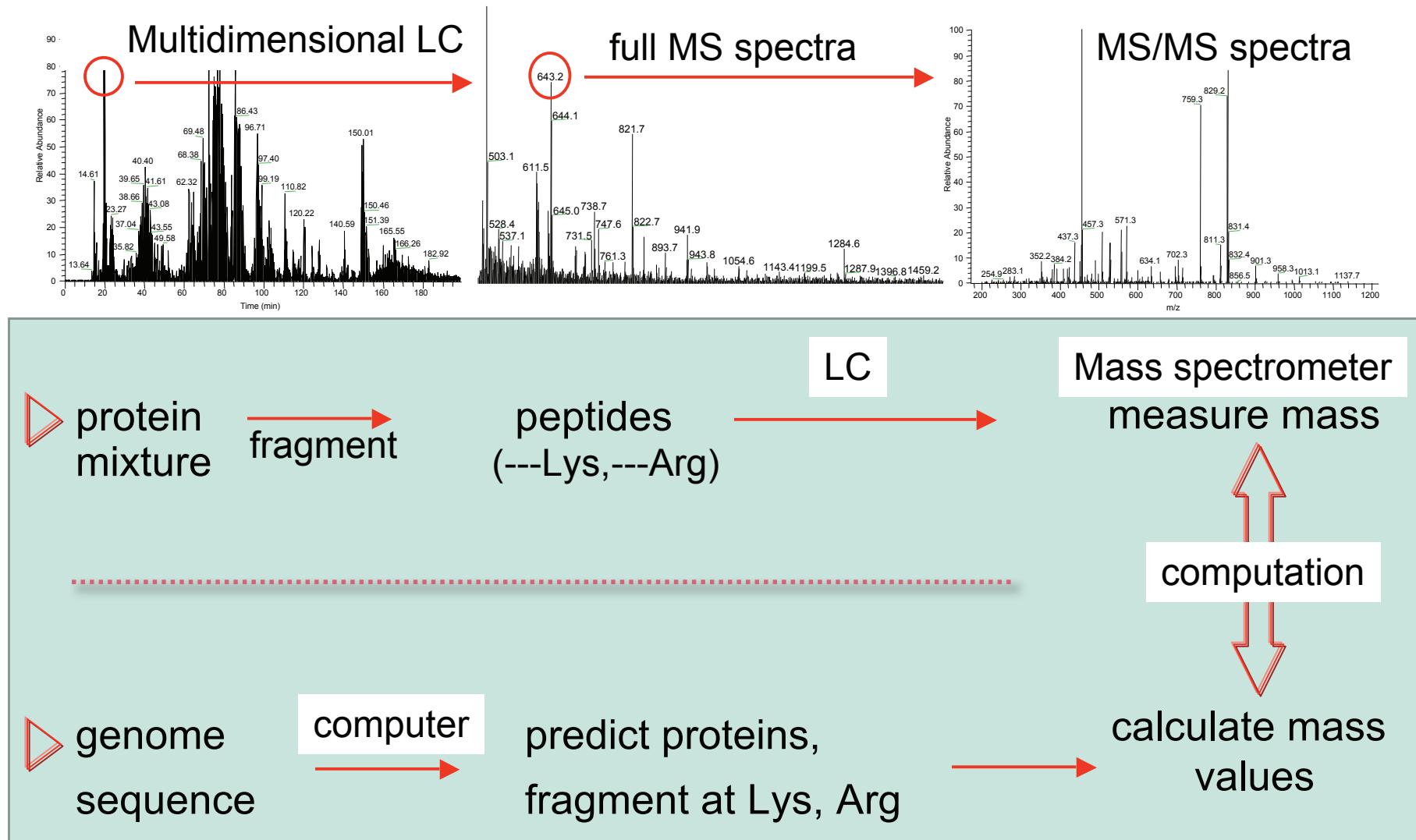
**Aim: to gain a greater understanding of the microbial community and its interactions with the subsurface environment during stimulated bioreduction.**

Community proteomic data provides information about:

1. microbial community composition at the strain level
2. protein expression



# GENOMIC DATA ENABLE PROTEOMIC ANALYSES:



Cytochrome 579

MNKWAGAVLGTVTLGLLSATAYS AELDILKPNRVPADQIAAAKAMKPPFPVTA  
AVIAKGKEVFNGAGTCYTCHVGKK **GDGPGAAGMDPSPR** FTNHQFDQVRTAGE  
MVWWVSNGSPLQPAMVGFVSAGITDKQ AWEAVMYERSLGCGGDMDC . . . . .

# Community proteogenomic analysis using isolate genomes

## **Geobacter species in proteomic search parameters:**

*G. uraniireducens*  
*G. lovleyi*  
*G. metallireducens*  
*G. sulfurreducens*  
*G. FRC-32*  
*G. bemandjiensis*  
*G. M-21*



Fairly similar evolutionary distances

Close relatives

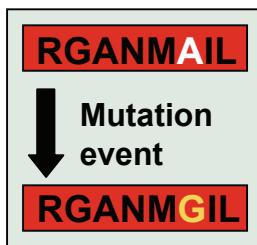
	M-21	Bemidjiensis	Metallireducens	Sulfurreducens	Uraniiireducens	FRC-32	Lovleyi
<b>M-21</b>	-	94%	65%	64%	67%	66%	60%
<b>Bemidjiensis</b>	<b>94%</b>	-	65%	64%	67%	66%	60%
<b>Metallireducens</b>	<b>65%</b>	<b>65%</b>	-	76%	68%	67%	62%
<b>Sulfurreducens</b>	<b>64%</b>	<b>64%</b>	<b>76%</b>	-	67%	66%	62%
<b>Uraniiireducens</b>	<b>67%</b>	<b>67%</b>	<b>68%</b>	<b>67%</b>	-	74%	61%
<b>FRC-32</b>	<b>66%</b>	<b>66%</b>	<b>67%</b>	<b>66%</b>	<b>74%</b>	-	61%
<b>Lovleyi</b>	<b>60%</b>	<b>60%</b>	<b>62%</b>	<b>62%</b>	<b>61%</b>	<b>61%</b>	-

Average ortholog % aa similarity

Questions:

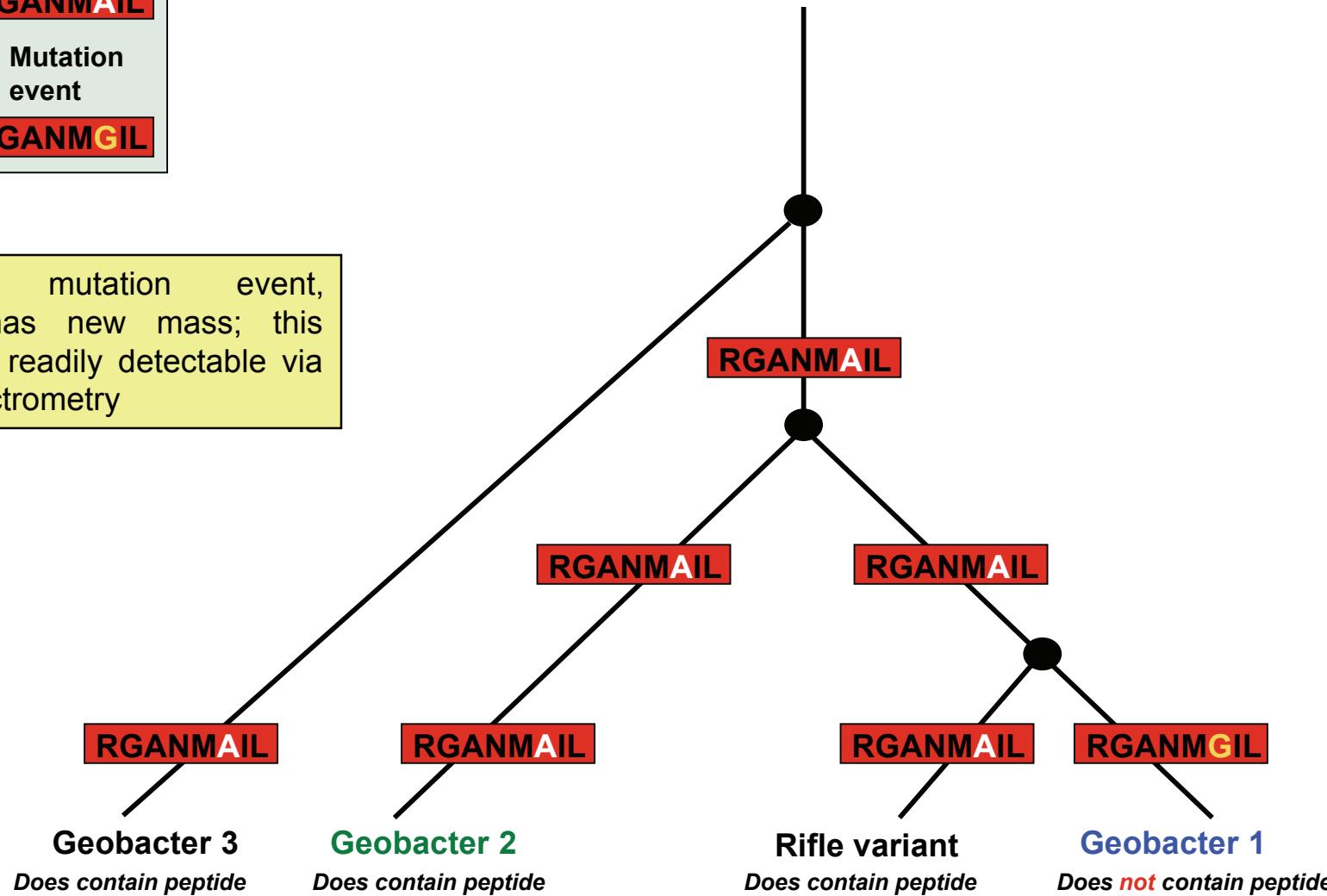
- are communities dominated by one strain / species or several?
- which isolate is the dominant strain / species most closely related to?

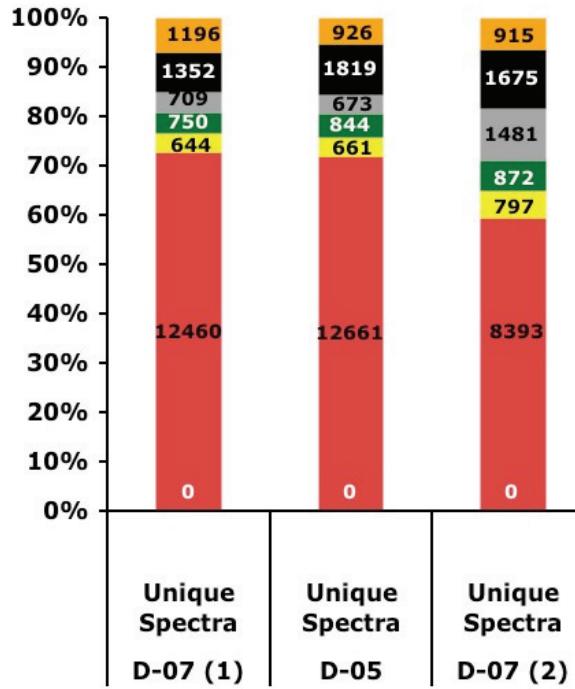
**Geobacter 1** ELAQLSACGVLPVGDSMEEIFEAVKYTALIHSGGGTGFSSRLRPANDVVMSTTGISSGPLSFMRVDVATETIKQGGTRRGANMGIL  
**Geobacter 2** ELGQLSACFVLPVGDSMEEIFESVKYTALIHSGGGTGFSSRLRPANDVVMSTTGISSGPLSFMRVDVATETIKQGGTRRGANMAIL  
**Geobacter 3** ELGQLSACFVLPVGDSMEEIFDAVKYTALIHSGGGTGFSSRLRPANDVVRSTSGISSGPISFMRVFDAAETEIKQGGTRRGANMAIL



## Common *Geobacter* ancestor

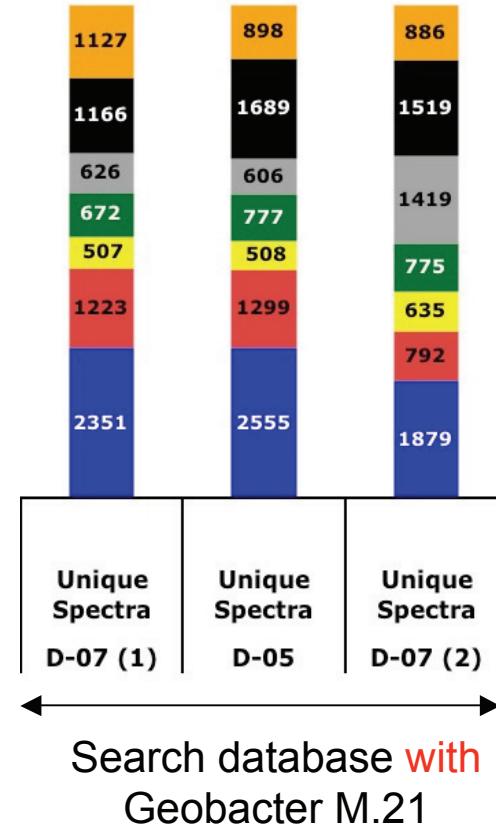
Following mutation event, peptide has new mass; this change is readily detectable via mass spectrometry





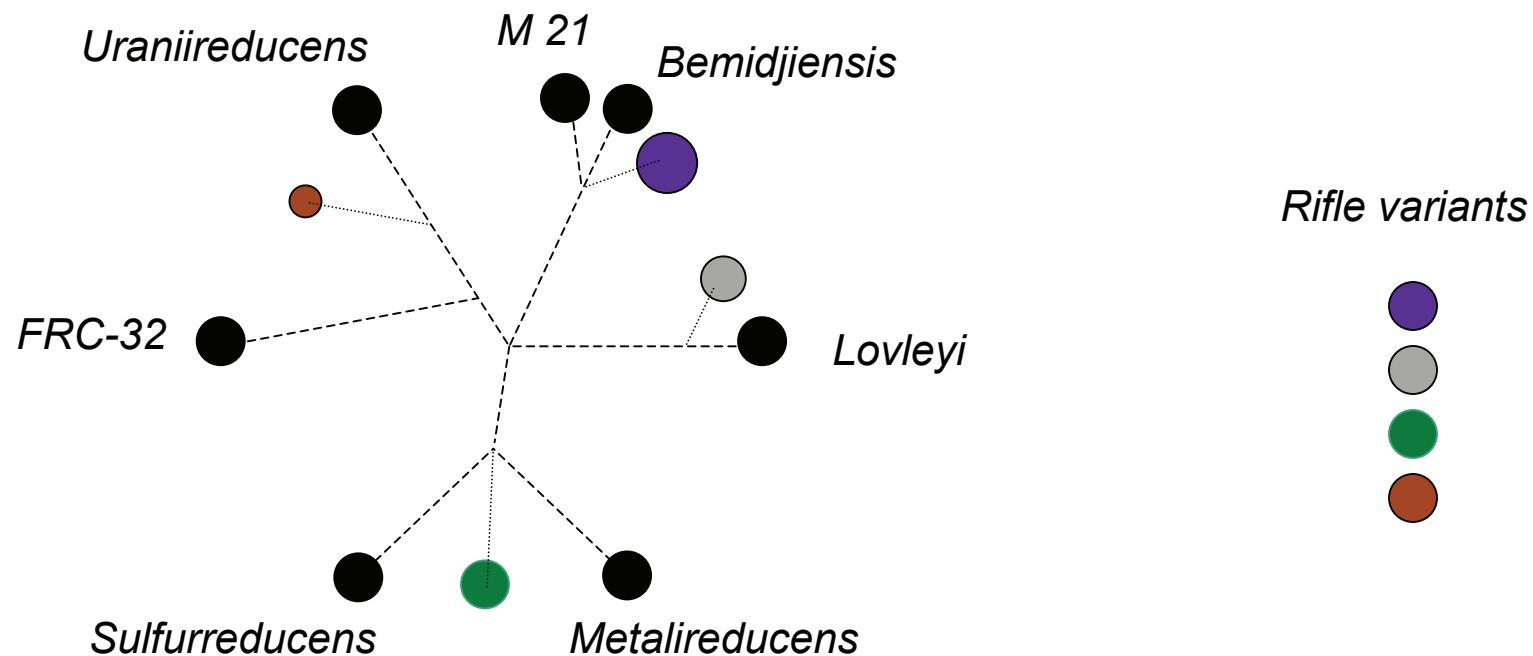
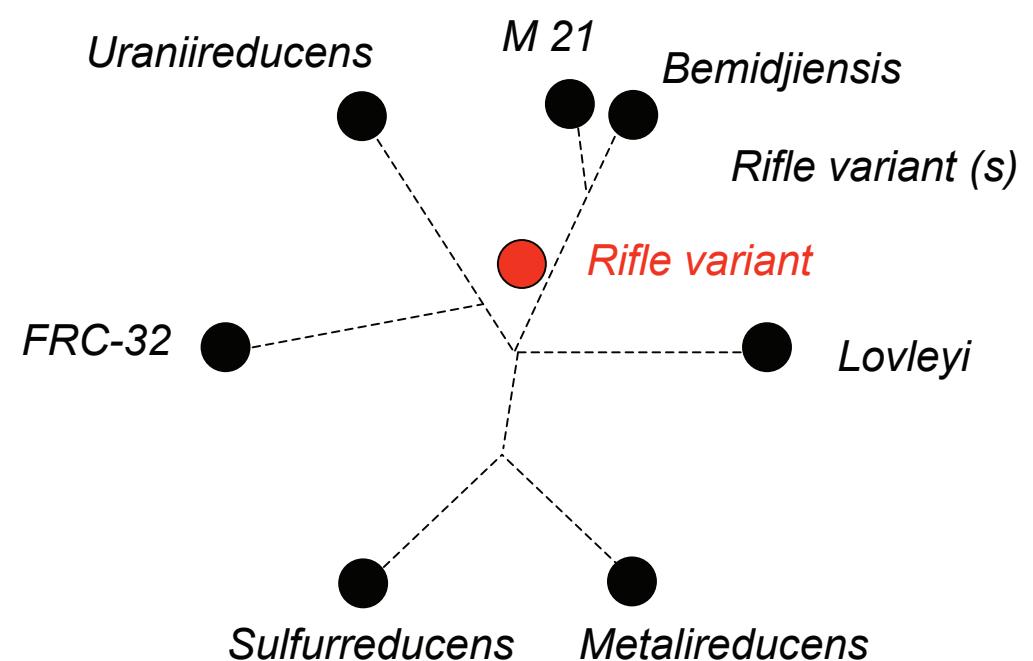
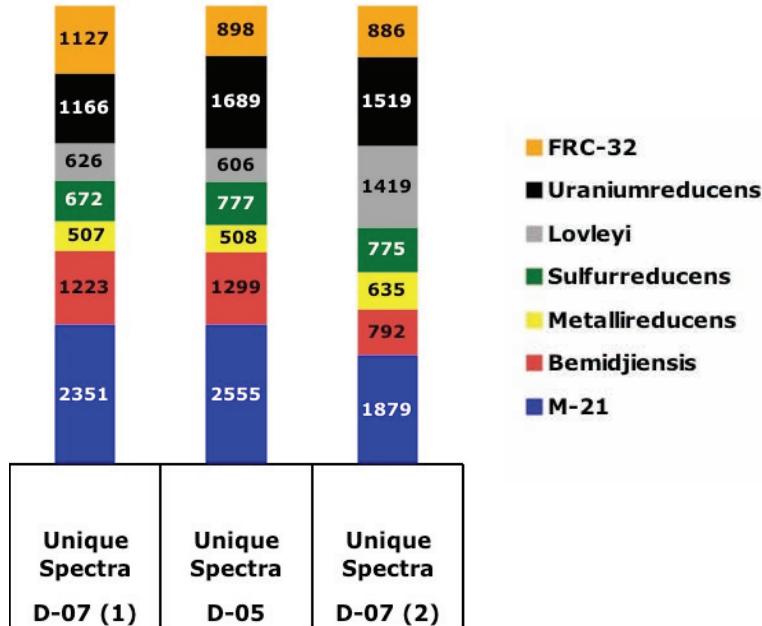
Most peptides derive from *G. bemiidjiensis*-like strain or strains

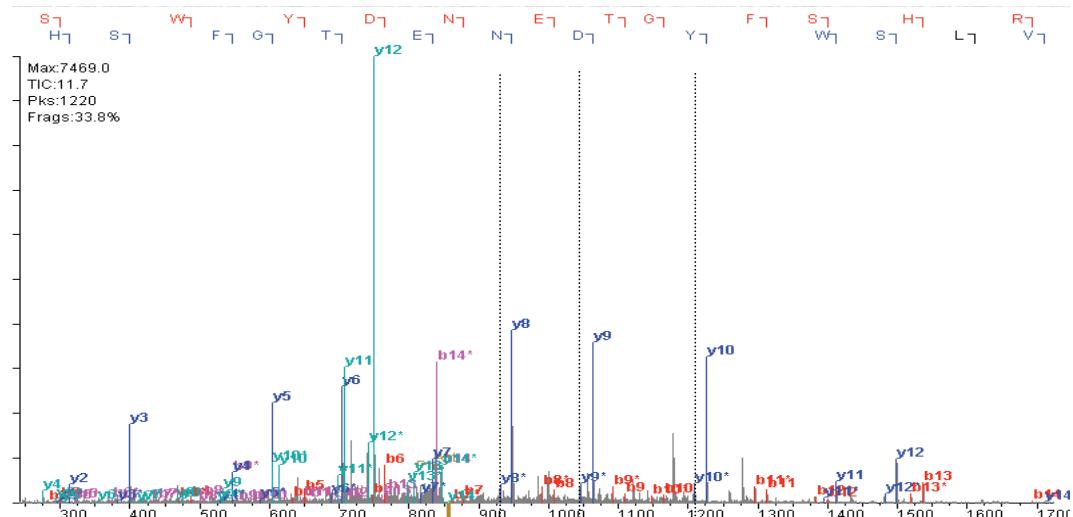
- FRC-32
- Uraniumreducens
- Lovleyi
- Sulfurreducens
- Metallireducens
- Bemidjiensis
- M-21



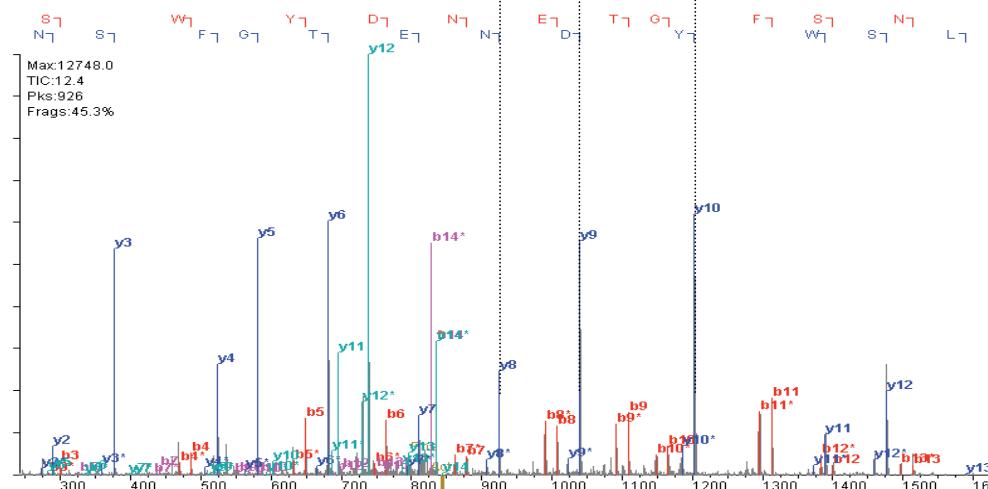
The addition of M-21 provides higher resolution of the actual strain or strains present

In theory, all unique spectra could be generated by one *Geobacter* species or derive from multiple species





K.VLSWYDNETGFS**HR**.V  
PPM: 1.4739  
 $\Delta$  AMU: 0.0025



K.VLSWYDNETGFS**NR**.V  
PPM: 2.4307  
 $\Delta$  AMU: 0.0041

Peptide from glyceraldehyde-3-phosphate dehydrogenase

# Proteogenomics with isolates

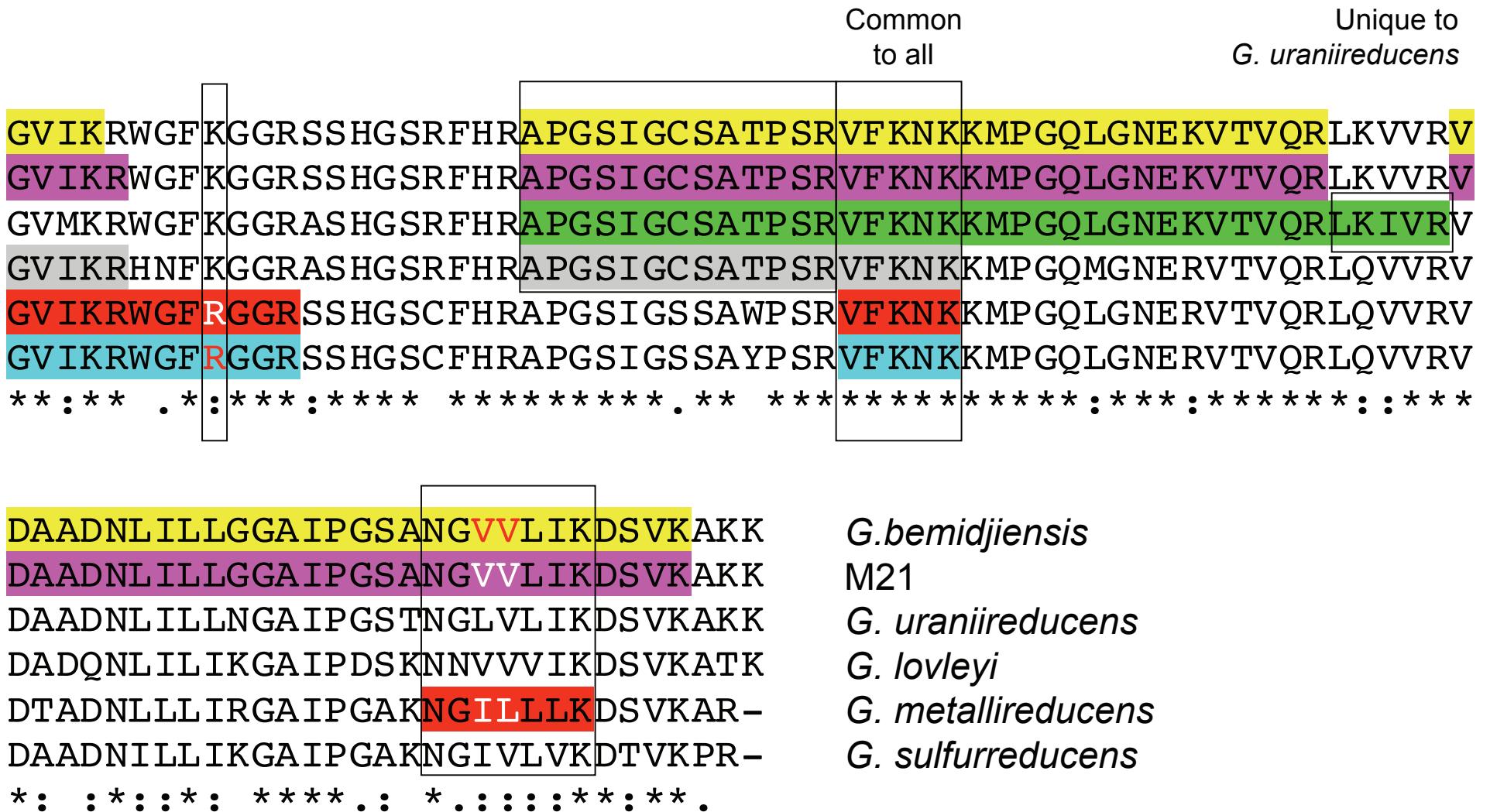
isolate 1	DTYKPEAQGEF <b>P</b> RNGETVIGRIGGIFAGKGMIHPQ <b>M</b> ATMVARVFL <b>A</b> TK....
isolate 2	DTYKPEAQGEF <b>A</b> RNGETVIGRIGGIFAGKGMIHPQ <b>F</b> ATMVARVFL <b>L</b> TK....
isolate 3	DTYKPEAQGEF <b>G</b> RNGETVIGRIGGIFAGKGMIHPQ <b>F</b> ATMVARVFL <b>T</b> TK....

Unique in all

Non-unique  
in all

Non-unique  
in 2 and 3

Unique in all

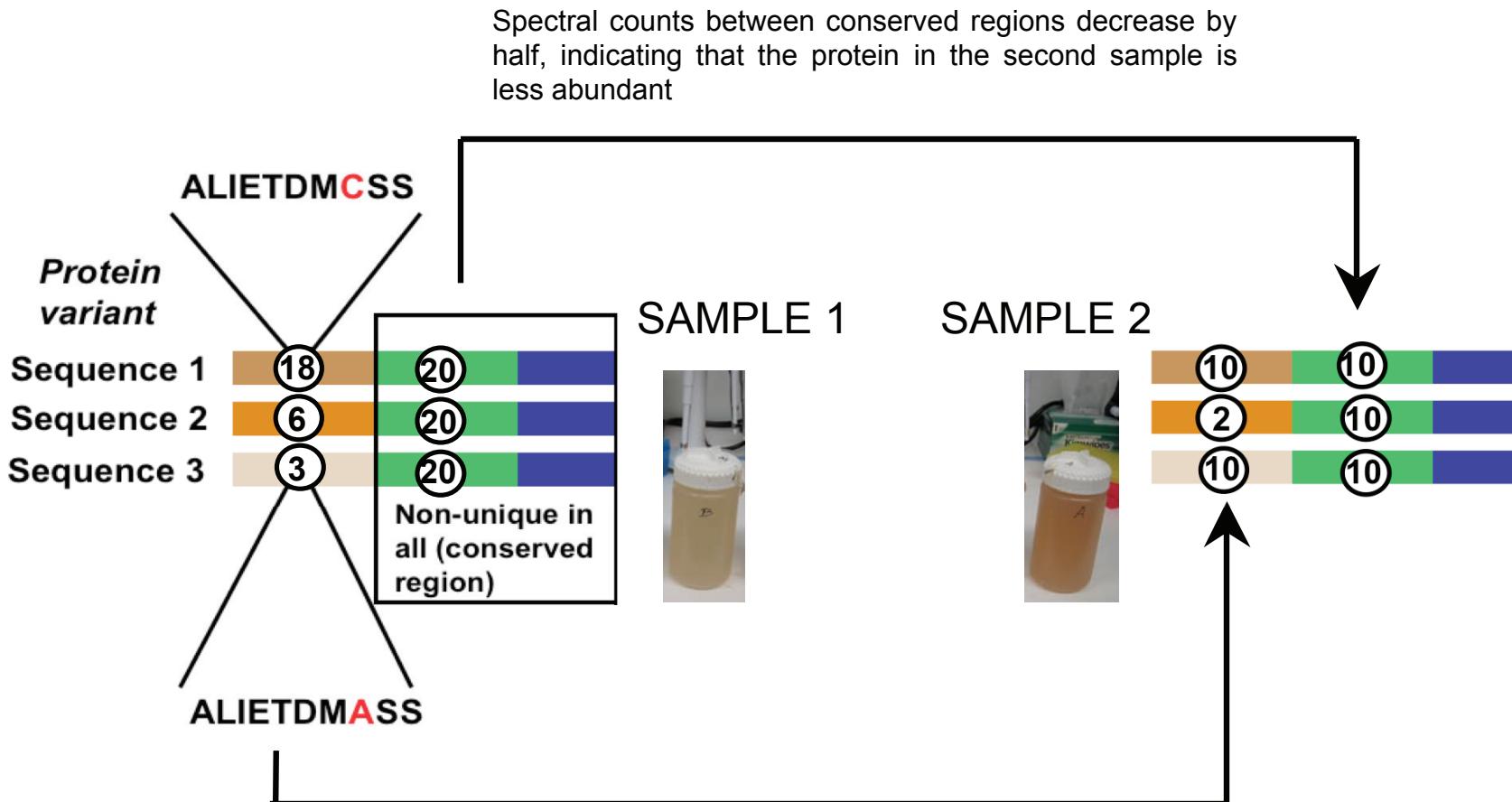


No strain is identical to any isolate genotype, multiple genotypes are present.  
 Genotypes are related to *G. bemiidjiensis*/M21 and *G. metallireducens*.

## Experimental data - Ribosomal protein L3, D-07(1)

Numerous co-existing unique spectra (circled) indicate the presence of multiple strain variants. This data is in agreement with SIP analysis which has detected 4 genomic *Geobacter* variants in similar samples

## Peptide mapping constrains species composition and protein abundance



Spectral counts for unique regions indicate that sample 1 is dominated by Sequence 1, with lesser amounts of 2 & 3.

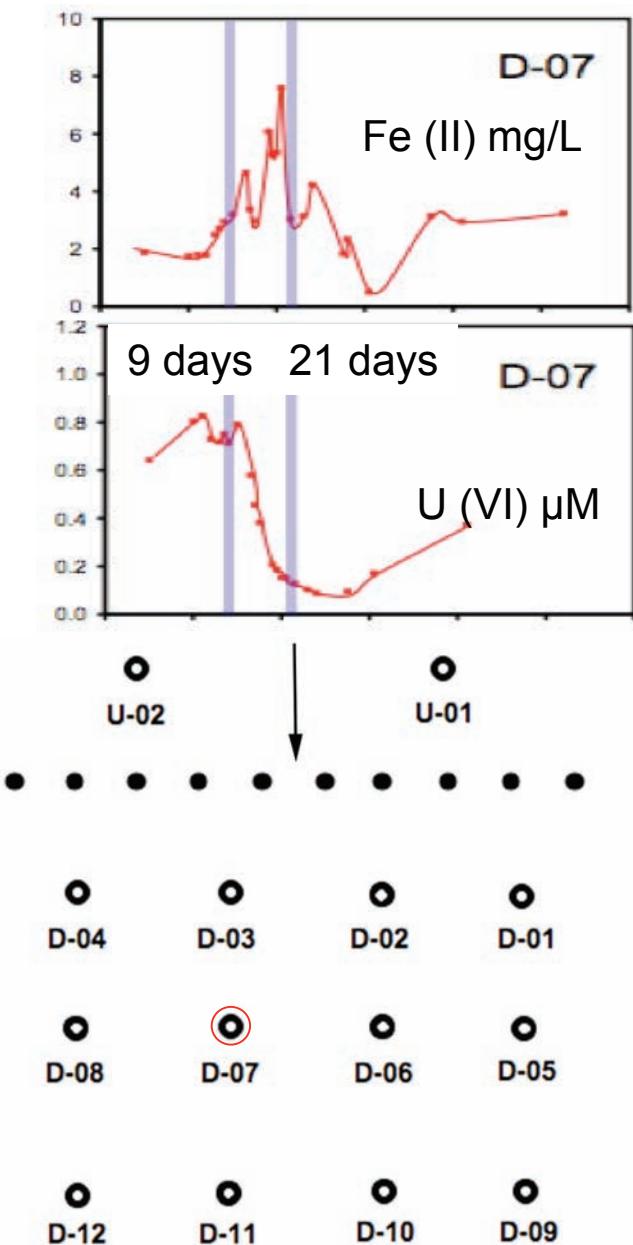
In sample 2, the community composition has changed, with 1 & 3 present in equal abundance, and a lesser amount of Sequence 2.

# Summer 2007: proteomic sampling during the biostimulation field experiment

500 L groundwater samples filtered using TFF

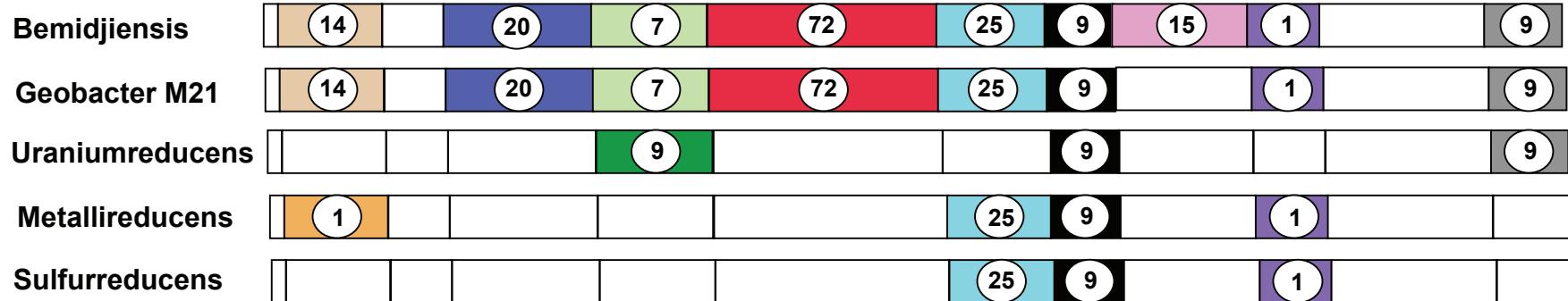


Downgradient  
wells  
2.5 m



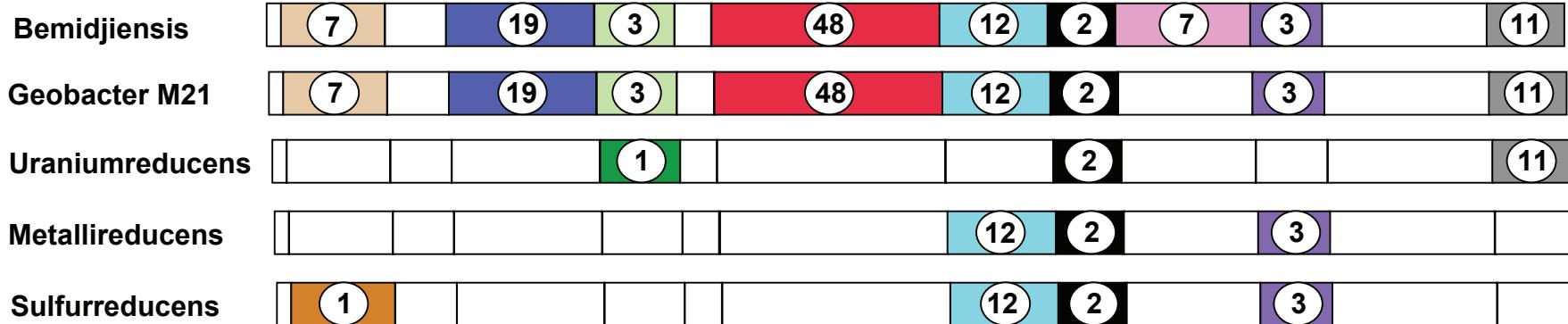
### Ribosomal protein S9 D-07(1)

Bemidjiensis            -MAVSFYATGKRKSSIARWIKPGNGEIIVNTKTLNDNYFGRETSKMVMQPLELTENVGKFDIFCTVKGGGDSQAGAIKHGITKALIEADDLRGTLKKAGFITRDSRIKERKKGKAARASFQFSKR  
 M21                    -MAVSFYATGKRKSSIARWIKPGNGEIIVNTKTLNDNYFGRETSKMVMQPLELTENVGKFDIFCTVKGGGDSQAGAIKHGITKALIEADGDLRGTLKKAGFITRDSRIKERKKGKAARASFQFSKR  
 Uraniumreducens    MAAASFYGTGKRKSSIARWVLKPGTGVITVNHKTLDEYFGRETSKMVVKQPLELTENMGKFDIYVTVCGGGDSQAGAIKHGITKALLEVDAALRGTLKKAGFVTRDSRIKERKKGKAARASFQFSKR  
 Metalli-reducens    MAAISYGTGKRKSSVARVWLKPGTGNIVINNKSIDDYFGRETSKMVVKQPLELVEKVGAFDIYVNVRGGGDSQAGAIKHGITKALLEVDVALRGTLKKAGFITRDSRIKERKKGKAARRSCQFSKR  
 Sulfurreducens       MAGISYGTGKRKSSVARVWLKPGTGAIVINNKPIDEYFGRETSKMVVKQPLELVEKVGAFDIYVNVRGGGDSQAGAIKHGITKALLEVDVALRGTLKKAGFITRDSRIKERKKGKAARRSCQFSKR



### Ribosomal protein S9 alignment D-07(2)

Bemidjiensis            -MAVSFYATGKRKSSIARWIKPGNGEIIVNTKTLNDNYFGRETSKMVMQPLELTENVGKFDIFCTVKGGGDSQAGAIKHGITKALIEADDLRGTLKKAGFITRDSRIKERKKGKAARASFQFSKR  
 M21                    -MAVSFYATGKRKSSIARWIKPGNGEIIVNTKTLNDNYFGRETSKMVMQPLELTENVGKFDIFCTVKGGGDSQAGAIKHGITKALIEADGDLRGTLKKAGFITRDSRIKERKKGKAARASFQFSKR  
 Uraniumreducens    MAAASFYGTGKRKSSIARWVLKPGTGVITVNHKTLDEYFGRETSKMVVKQPLELTENMGKFDIYVTVCGGGDSQAGAIKHGITKALLEVDAALRGTLKKAGFVTRDSRIKERKKGKAARASFQFSKR  
 Metalli-reducens    MAAISYGTGKRKSSVARVWLKPGTGNIVINNKSIDDYFGRETSKMVVKQPLELVEKVGAFDIYVNVRGGGDSQAGAIKHGITKALLEVDVALRGTLKKAGFITRDSRIKERKKGKAARRSCQFSKR  
 Sulfurreducens       MAGISYGTGKRKSSVARVWLKPGTGAIVINNKPIDEYFGRETSKMVVKQPLELVEKVGAFDIYVNVRGGGDSQAGAIKHGITKALLEVDVALRGTLKKAGFITRDSRIKERKKGKAARRSCQFSKR

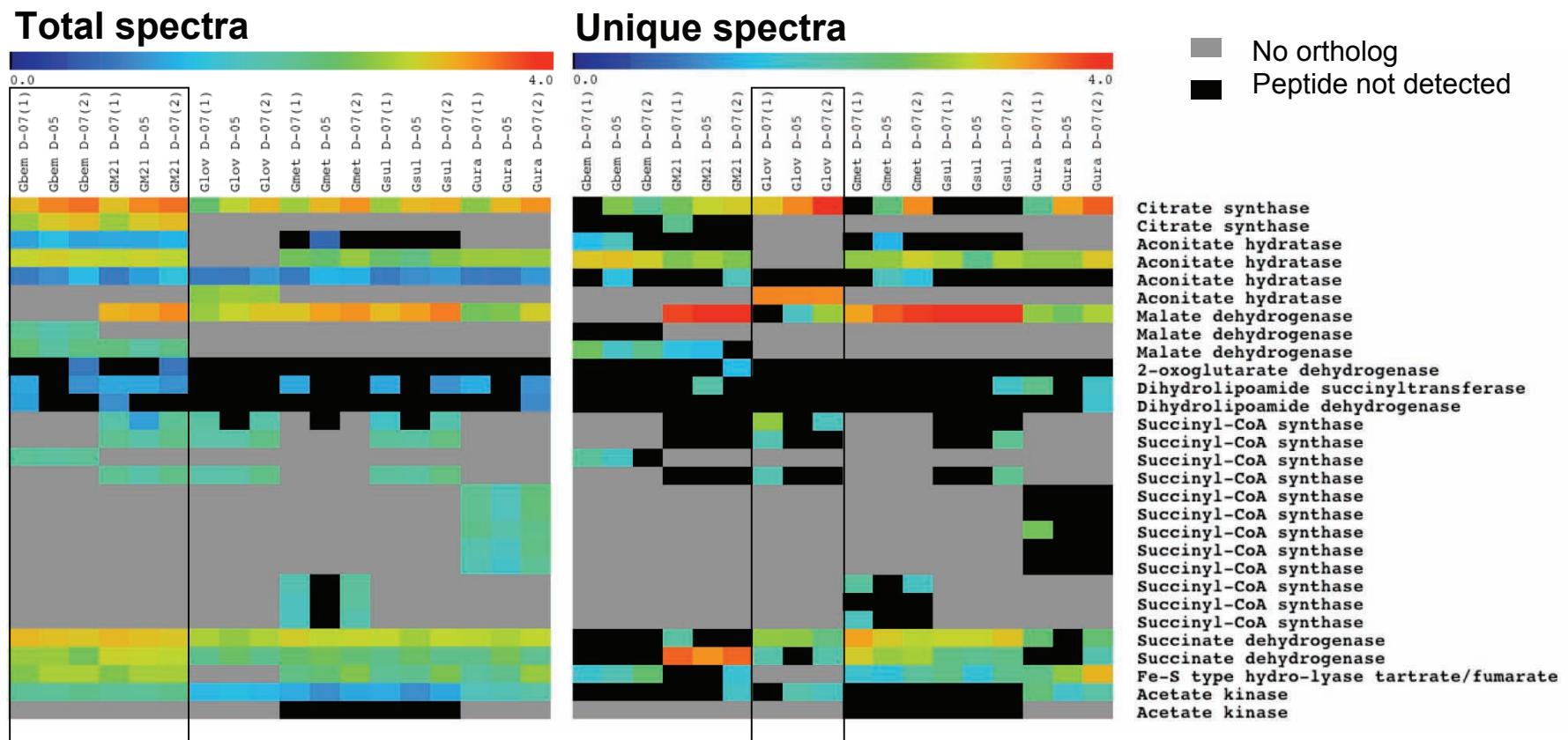


Species composition is similar, but abundance decreases in the second sample

All major TCA cycle proteins detected; dominance by Gbem / M21-like species

Unique peptides for TCA cycle, ATP synthase, NADH dehydrogenase proteins indicate activity of multiple *Geobacter* species

Changes in abundance levels over time indicate changes in species roles in the community (activity or number)



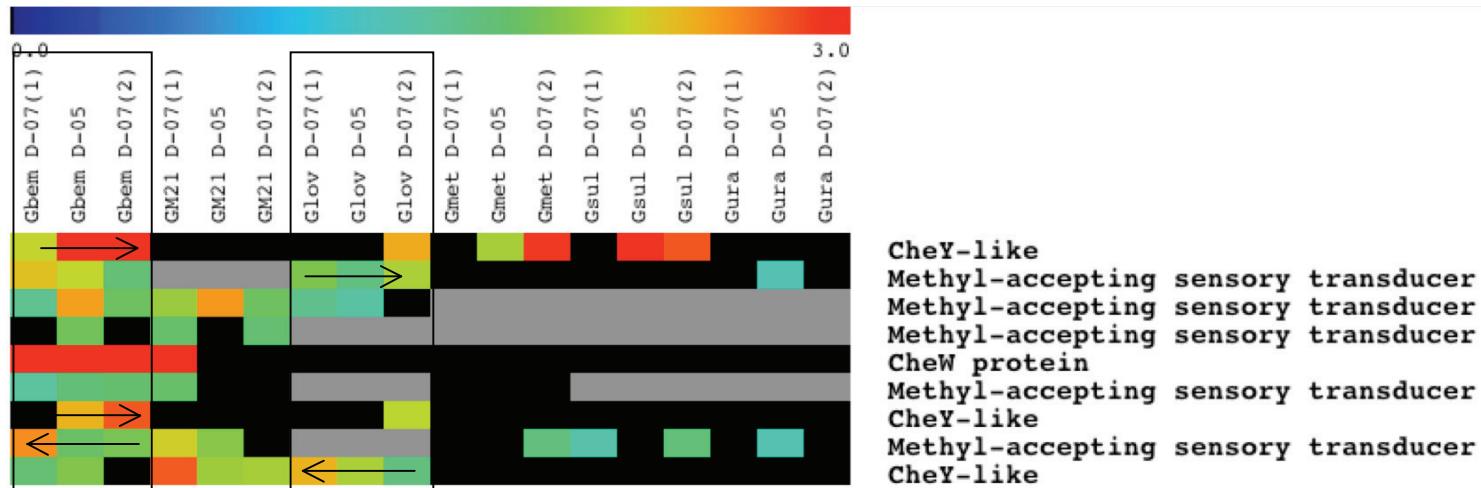
During the experiment, changes in the abundances of proteins involved in chemotaxis suggest that *Geobacter* species rapidly respond to changing environmental stimuli.

For example, across three samples :-

90 of the 140 histidine kinase/ response regulator /Che genes in *G. bemandjiensis* were identified

Similarly, *G. M-21* contains ~165 chemotaxis genes, of which over 80 were identified

Heat-map showing the normalized abundance of **unique spectra** for a selection of chemotaxis genes in 6 *Geobacter* species across the three samples (D-07(1), D-05 & D-07(2))

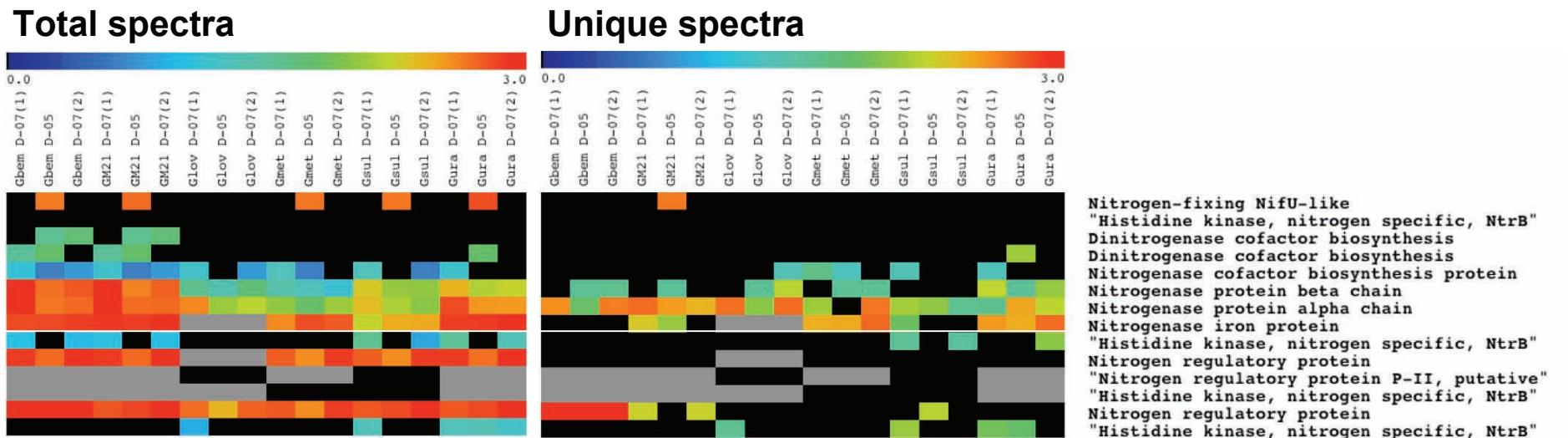


Presence of co-existing unique spectra indicates the activity of co-existing *Geobacter* strains

Expression of proteins involved in nitrogen fixation in all three samples by multiple co-existing *Geobacter* species

Peptide mapping indicates that abundance for the nitrogenase β chain is similar in D-07(1) and D-07(2), samples from early and mid biostimulation

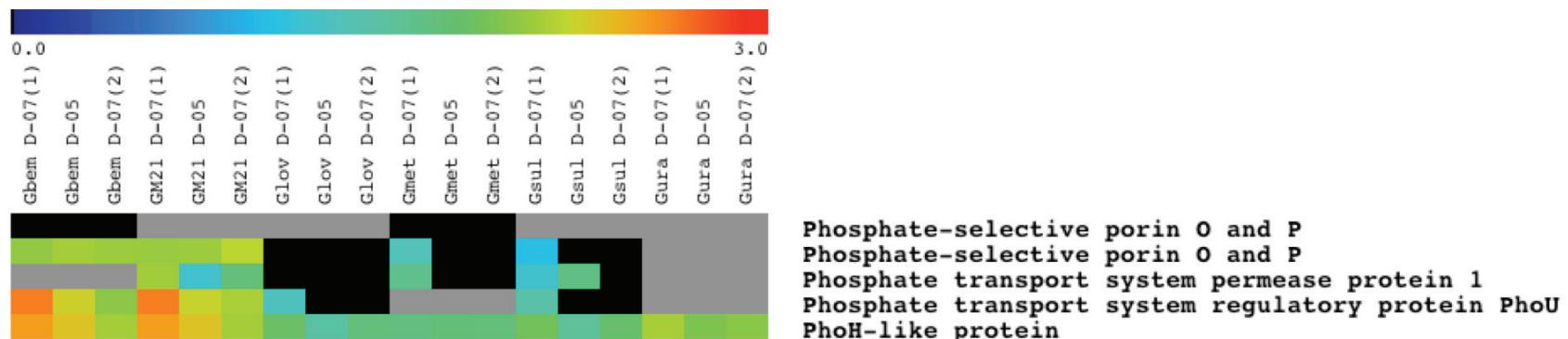
No detection of ammonium transporter proteins



Large numbers of conserved peptides, hence low numbers of unique spectra  
 (circled region shows few unique spectra relative to high numbers of total spectra)

## Phosphate acquisition

Range of proteins involved in P uptake identified (unique peptides for multiple species), however protein abundances appear relatively low.



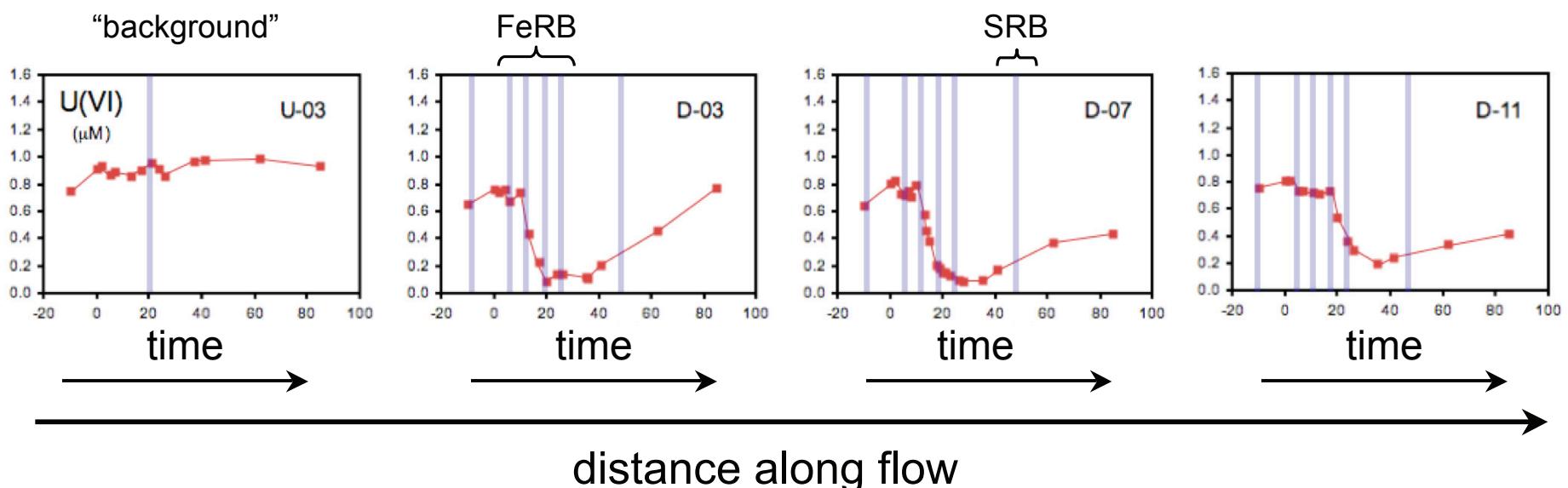
## Differentiating strain roles?

Only *G. uraniireducens* has, and expresses, a cluster of genes involved in the synthesis of macrolides and ketolides - intermediates in antibiotic production (or involved in electron shuttling)

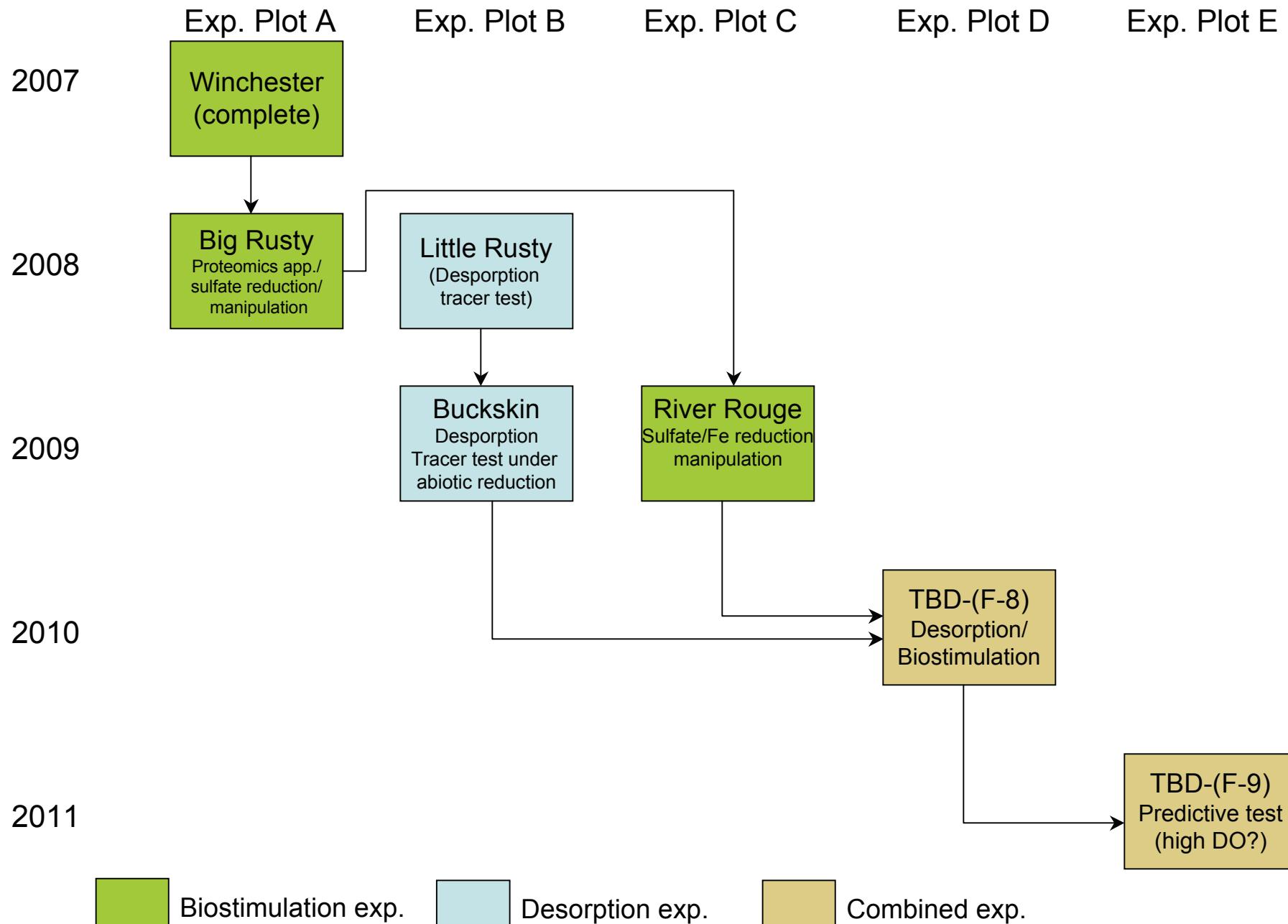
Gura_3094	NO		Erythronolide synthase( EC:2.3.1.94 )
Gura_3095	NO		beta-ketoacyl synthase
Gura_3096	NO		Erythronolide synthase( EC:2.3.1.94 )
Gura_3097	NO		beta-ketoacyl synthase
Gura_3098	NO		Mycocerosate synthase( EC:2.3.1.111 )

## 2008 “Big Rusty” field experiment:

- Using same flow cell, replicate 5 mM acetate injection to validate 2007 results
- Verify low volume (100 L) sampling approach to obtain sufficient biomass and minimize proteome changes
- Greatly expand spatiotemporal sampling during (i) iron reduction, (ii) optimal U-removal, and (iii) transition to sulfate reduction
- Obtain unamended “background” sample for comparison

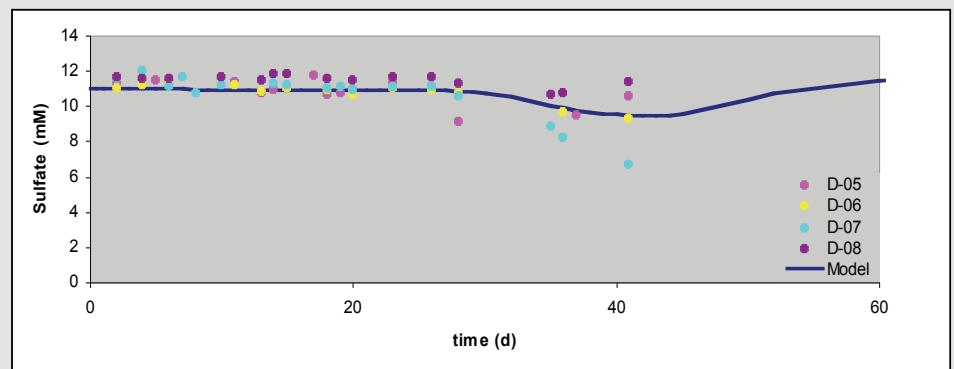
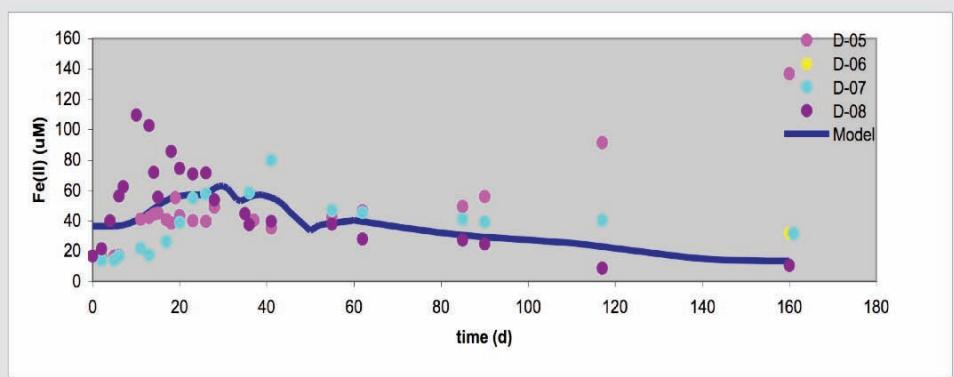
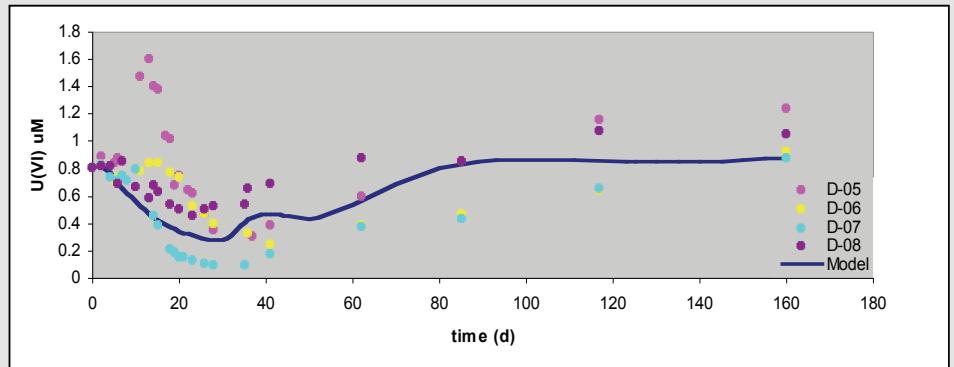


# Rifle IFC Field Experiment Schedule (Rev 4 April 08)



# Expected outcomes and modeling progress

- ▶ Mechanistic understanding of controls on U(VI) bioreduction
- ▶ Predictive modeling capability for both biostimulated and natural U(VI) bioreduction
- ▶ Linkage of desorption and bioreduction for U bioremediation
- ▶ Long-term monitoring of U-contaminated sites via biogeochemical sensors
- ▶ Above tools incorporated into DOE cleanup and monitoring decisions



See also *in silico* modeling-RTM poster  
(Scheibe, Lovley, Mahadevan, Fang,  
Long et al.)



*U.S. Department of Energy Office of Science*

**Environmental Remediation Sciences Program**

# **Acknowledgement: Rifle Integrated Field Challenge Site**

- ▶ Funding for the Rifle IFC is provided by the U.S. Department of Energy, Office of Science, Biological and Environmental Research, Environmental Remediation Sciences Division